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SUBSTITUTED AMINOETHERS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application Serial No. 60/409,565, filed September 10, 2002, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to substituted aminoethers and to such compounds that are useful in treatment of Alzheimer's disease and similar diseases, more specifically it relates to such compounds that inhibit β-secretase, an enzyme that cleaves amyloid precursor protein to produce Aβ peptide, a major component of the amyloid plaques found in the brains of Alzheimer's sufferers.

Description of the Related Art

Alzheimer's disease (AD) is a progressive degenerative disease of the brain primarily associated with aging. Clinical presentation of AD is characterized by loss of memory, cognition, reasoning, judgement, and orientation. As the disease progresses, motor, sensory, and linguistic abilities are also affected until there is global impairment of multiple cognitive functions. These cognitive losses occur gradually, but typically lead to severe impairment and eventual death in the range of four to twelve years.

Alzheimer's disease is characterized by two major pathologic observations in the brain: neurofibrillary tangles and beta amyloid (or neuritic) plaques, comprised predominantly of an aggregate of a peptide fragment know as A beta. Individuals with AD exhibit characteristic beta-amyloid deposits in the brain (beta amyloid plaques) and in cerebral blood vessels (beta amyloid angiopathy) as well as neurofibrillary tangles. Neurofibrillary tangles occur not

only in Alzheimer's disease but also in other dementia-inducing disorders. On autopsy, large numbers of these lesions are generally found in areas of the human brain important for memory and cognition.

Smaller numbers of these lesions in a more restricted anatomical distribution are found in the brains of most aged humans who do not have clinical AD. Amyloidogenic plaques and vascular amyloid angiopathy also characterize the brains of individuals with Trisomy 21 (Down's Syndrome), Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-Type (HCHWA-D), and other neurogenerative disorders. Beta-amyloid is a defining feature of AD, now believed to be a causative precursor or factor in the development of disease. Deposition of A beta in areas of the brain responsible for cognitive activities is a major factor in the development of AD. Beta-amyloid plaques are predominantly composed of amyloid beta peptide (A beta, also sometimes designated betaA4). A beta peptide is derived by proteolysis of the amyloid precursor protein (APP) and is comprised of 39-42 amino acids. Several proteases called secretases are involved in the processing of APP.

Cleavage of APP at the N-terminus of the A beta peptide by beta-secretase and at the C-terminus by one or more gamma-secretases constitutes the beta-amyloidogenic pathway, i.e. the pathway by which A beta is formed. Cleavage of APP by alpha-secretase produces alpha-sAPP, a secreted form of APP that does not result in beta-amyloid plaque formation. This alternate pathway precludes the formation of A beta peptide. A description of the proteolytic processing fragments of APP is found, for example, in U.S. Patent Nos. 5,441,870; 5,721,130; and 5,942,400.

An aspartyl protease has been identified as the enzyme responsible for processing of APP at the beta-secretase cleavage site. The beta-secretase enzyme has been disclosed

using varied nomenclature, including BACE, Asp, am Mamepsin. See, for example, Sindha et.al., 1999, *Nature* 402:537-554 (p501) and published PCT application WO00/17369.

Several lines of evidence indicate that progressive cerebral deposition of beta-amyloid peptide (A beta) plays a seminal role in the pathogenesis of AD and can precede cognitive symptoms by years or decades. See, for example, Selkoe, 1991, *Neuron* 6:487. Release of A beta from neuronal cells grown in culture and the presence of A beta in cerebrospinal fluid (CSF) of both normal individuals and AD patients has been demonstrated. See, for example, Seubert et al., 1992, *Nature* 359:325-327.

It has been proposed that A beta peptide accumulates as a result of APP processing by beta˜secretase, thus inhibition of this enzyme's activity is desirable for the treatement of AD. *In vivo* processing of APP at the beta-secretase cleavage site is thought to be a rate-limiting step in A beta production, and is thus a therapeutic target for the treatment of AD. See for example, Sabbagh, M., et al., 1997, *Alz. Dis. Rev.* 3, 1-19.

BACE1 knockout mice fail to produce A beta, and present a normal phenotype. When crossed with transgenic mice that overexpress APP, the progeny show reduced amounts of A beta in brain extracts as compared with control animals (Luo et.al., 2001 *Nature Neuroscience* 4:231-232). This evidence further supports the proposal that inhibition of beta-secretase activity and reduction of A beta in the brain provides a therapeutic method for the treatment of AD and other beta amyloid disorders.

Published PCT application WO00/47618 entitled "Beta-Secretase Enzyme Compositions and Methods" identifies the beta-secretase enzyme and methods of its use. This publication also discloses oligopeptide inhibitors that bind the enzyme's active site and are useful in affinity column

purification of the enzyme. In addition, WO00/77030 discloses tetrapeptide inhibitors of beta-secretase activity that are based on a statine molecule

Various pharmaceutical agents have been proposed for the treatment of Alzheimer's disease but without any real success. US Patent 5,175,281 discloses 21-aminosteroids as being useful for treating Alzheimer's disease. US Patent 5,502,187 discloses bicyclic heterocyclic amines as being useful for treating Alzheimer's disease.

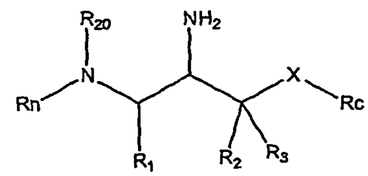
The hydroxyethylamine "nucleus" or isostere, of which the compounds of the invention is a truncated analog, has been used with success in the area of HIV protease inhibition. Many of these hydroxyethylamine compounds are known as well as how to make them. See for example, *J. Am. Chem. Soc.*, 93, 288-291 (1993), *Tetrahedron Letters*, 28(45) 5569-5572 (1987), *J. Med. Chem.*, 38(4), 581-584 (1994), *Tetrahedron Letters*, 38(4), 619-620 (1997). European Patents, numbers 702 004, 678 503, 678 514, 678 503 and 716077 by Maibaum, et al. are directed to similar isosteric strategies directed at renin inhibition. See also, U.S. Pat. Nos. 5,606,078 and 5,559,111, both to Goschke, et. al.; 5,719,141, to Rasetti, et. al.; and 5,641,778, to Maibaum, et. al.

At present there are no effective treatments for halting, preventing, or reversing the progression of Alzheimer's disease. Therefore, there is an urgent need for pharmaceutical agents capable of slowing the progression of Alzheimer's disease and/or preventing it in the first place.

Compounds that are effective inhibitors of beta-secretase, that inhibit beta-secretase-mediated cleavage of APP, that are effective inhibitors of A beta production, and/or are effective to reduce amyloid beta deposits or plaques, are needed for the treatment and prevention of disease characterized by amyloid beta deposits or plaques, such as AD.

SUMMARY OF THE INVENTION

In a broad aspect, the invention provides compounds of formula (I):



(I)

or pharmaceutically acceptable salts or esters thereof;

wherein X is O, S, NR_{20}, or $NR_{20}NR_{20}$;

wherein each R_{20} is H, C_{1-6} alkyl or alkenyl, C_{1-6} haloalkyl or C_{4-7} cycloalkyl;

wherein R_1 is $-(CH_2)_{1-2}-S(O)_{0-2}-(C_1-C_6$ alkyl), or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, =O, -SH, -C≡N, $-CF_3$, $-C_1-C_3$ alkoxy, amino, mono- or dialkylamino, -N(R)C(O)R'-, -OC(=O)-amino and -OC(=O)-mono- or dialkylamino, or

C_2-C_6 alkenyl or C_2-C_6 alkynyl, each of which is optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, amino, and mono- or dialkylamino, or

aryl, heteroaryl, heterocyclyl, $-C_1-C_6$ alkyl-aryl, $-C_1-C_6$ alkyl-heteroaryl, or $-C_1-C_6$ alkyl-heterocyclyl, where the ring portions of each are optionally substituted with 1, 2, 3, or 4 groups independently selected from halogen, -OH, -SH, -C≡N, $-NR_{105}R'_{105}$, $-CO_2R$, -N(R)COR', or $-N(R)SO_2R'$, $-C(=O)-(C_1-C_4)$ alkyl, $-SO_2$-amino, $-SO_2$-mono or dialkylamino, -C(=O)-amino, -C(=O)-mono or dialkylamino, $-SO_2-(C_1-C_4)$ alkyl, or

C_1-C_6 alkoxy optionally substituted with 1, 2, or 3 groups which are independently selected from halogen, or

C_3-C_7 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, amino, $-C_1$-C_6 alkyl and mono- or dialkylamino, or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, $-CF_3$, $-C_1$-C_3 alkoxy, amino, mono- or dialkylamino and $-C_1$-C_3 alkyl, or

C_2-C_{10} alkenyl or C_2-C_{10} alkynyl each of which is optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, amino, C_1-C_6 alkyl and mono- or dialkylamino; and the heterocyclyl group is optionally further substituted with oxo;

R and R' independently are hydrogen, C_1-C_{10} alkyl, C_1-C_{10} alkylaryl or C_1-C_{10} alkylheteroaryl;

wherein R_C is hydrogen, $-(CR_{245}R_{250})_{0-4}$-aryl, $-(CR_{245}R_{250})_{0-4}$-heteroaryl, $-(CR_{245}R_{250})_{0-4}$-heterocyclyl, $-(CR_{245}R_{250})_{0-4}$-aryl-heteroaryl, $-(CR_{245}R_{250})_{0-4}$-aryl-heterocyclyl, $-(CR_{245}R_{250})_{0-4}$-aryl-aryl, $-(CR_{245}R_{250})_{0-4}$-heteroaryl-aryl, $-(CR_{245}R_{250})_{0-4}$-heteroaryl-heterocyclyl, $-(CR_{245}R_{250})_{0-4}$-heteroaryl-heteroaryl, $-(CR_{245}R_{250})_{0-4}$-heterocyclyl-heteroaryl, $-(CR_{245}R_{250})_{0-4}$-heterocyclyl-heterocyclyl, $-(CR_{245}R_{250})_{0-4}$-heterocyclyl-aryl, $-[C(R_{255})(R_{260})]_{1-3}$-CO-N-$(R_{255})_2$, $-CH(aryl)_2$, $-CH(heteroaryl)_2$, $-CH(heterocyclyl)_2$, -CH(aryl)(heteroaryl), $-(CH_2)_{0-1}$-CH($(CH_2)_{0-6}$-OH)-$(CH_2)_{0-1}$-aryl, $-(CH_2)_{0-1}$-CH($(CH_2)_{0-6}$-OH-$(CH_2)_{0-1}$-heteroaryl, -CH(-aryl or -heteroaryl)-CO-O(C_1-C_4 alkyl), -CH($-CH_2$-OH)-CH(OH)-phenyl-NO_2, (C_1-C_6 alkyl)-O-(C_1-C_6 alkyl)-OH; $-CH_2$-NH-CH_2-CH(-O-CH_2-CH_3)$_2$, $-(CH_2)_{0-6}$-C(=NR_{235})($NR_{235}R_{240}$), or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from the group consisting of

R_{205}, $-OC{=}ONR_{235}R_{240}$, $-S(=O)_{0-2}(C_1-C_6$ alkyl), -SH, $-NR_{235}C{=}ONR_{235}R_{240}$, $-C{=}ONR_{235}R_{240}$, and $-S(=O)_2NR_{235}R_{240}$, or

$-(CH_2)_{0-3}-(C_3-C_8)$ cycloalkyl wherein the cycloalkyl is optionally substituted with 1, 2, or 3 groups independently selected from the group consisting of R_{205}, $-CO_2H$, and $-CO_2-(C_1-C_4$ alkyl), or

cyclopentyl, cyclohexyl, or cycloheptyl ring fused to aryl, heteroaryl, or heterocyclyl wherein one, two or three carbons of the cyclopentyl, cyclohexyl, or cycloheptyl is optionally replaced with a heteroatom independently selected from NH, NR_{215}, O, or $S(=O)_{0-2}$, and wherein the cyclopentyl, cyclohexyl, or cycloheptyl group can be optionally substituted with one or two groups that are independently R_{205}, =O, $-CO-NR_{235}R_{240}$, or $-SO_2-(C_1-C_4$ alkyl), or

C_2-C_{10} alkenyl or C_2-C_{10} alkynyl, each of which is optionally substituted with 1, 2, or 3 R_{205} groups, wherein

each aryl and heteroaryl is optionally substituted with 1, 2, or 3 R_{200}, and wherein each heterocyclyl is optionally substituted with 1, 2, 3, or 4 R_{210};

R_{200} at each occurrence is independently selected from -OH, $-NO_2$, halogen, $-CO_2H$, C≡N, $-(CH_2)_{0-4}-CO-NR_{220}R_{225}$, $-(CH_2)_{0-4}-CO-(C_1-C_{12}$ alkyl), $-(CH_2)_{0-4}-CO-(C_2-C_{12}$ alkenyl), $-(CH_2)_{0-4}-CO-(C_2-C_{12}$ alkynyl), $-(CH_2)_{0-4}-CO-(C_3-C_7$ cycloalkyl), $-(CH_2)_{0-4}$-CO-aryl, $-(CH_2)_{0-4}$-CO-heteroaryl, $-(CH_2)_{0-4}$-CO-heterocyclyl, $-(CH_2)_{0-4}-CO-O-R_{215}$, $-(CH_2)_{0-4}-SO_2-NR_{220}R_{225}$, $-(CH_2)_{0-4}-SO-(C_1-C_8$ alkyl), $-(CH_2)_{0-4}-SO_2-(C_1-C_{12}$ alkyl), $-(CH_2)_{0-4}-SO_2-(C_3-C_7$ cycloalkyl), $-(CH_2)_{0-4}-N(H$ or $R_{215})-CO-O-R_{215}$, $-(CH_2)_{0-4}-N(H$ or $R_{215})-CO-N(R_{215})_2$, $-(CH_2)_{0-4}-N-CS-N(R_{215})_2$, $-(CH_2)_{0-4}-N(-H$ or $R_{215})-CO-R_{220}$, $-(CH_2)_{0-4}-NR_{220}R_{225}$, $-(CH_2)_{0-4}-O-CO-(C_1-C_6$ alkyl), $-(CH_2)_{0-4}-O-P(O)-(OR_{240})_2$, $-(CH_2)_{0-4}-O-CO-N(R_{215})_2$, $-(CH_2)_{0-4}-O-CS-N(R_{215})_2$, $-(CH_2)_{0-4}-O-(R_{215})$, $-(CH_2)_{0-4}-O-(R_{215})-COOH$, $-(CH_2)_{0-4}-S-(R_{215})$, $-(CH_2)_{0-4}-$

O-(C_1-C_6 alkyl optionally substituted with 1, 2, 3, or 5 -F), C_3-C_7 cycloalkyl, -$(CH_2)_{0-4}$-N(H or R_{215})-SO_2-R_{220}, -$(CH_2)_{0-4}$- C_3-C_7 cycloalkyl, or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 R_{205} groups, or

C_2-C_{10} alkenyl or C_2-C_{10} alkynyl, each of which is optionally substituted with 1 or 2 R_{205} groups, wherein

the aryl and heteroaryl groups at each occurrence are optionally substituted with 1, 2, or 3 groups that are independently R_{205}, R_{210}, or

C_1-C_6 alkyl substituted with 1, 2, or 3 groups that are independently R_{205} or R_{210}, and wherein

the heterocyclyl group at each occurrence is optionally substituted with 1, 2, or 3 groups that are independently R_{210};

R_{205} at each occurrence is independently selected from C_1-C_6 alkyl, halogen, -OH, -O-phenyl, -SH, -C≡N, -CF_3, C_1-C_6 alkoxy, NH_2, NH(C_1-C_6 alkyl) or N-(C_1-C_6 alkyl)(C_1-C_6 alkyl);

R_{210} at each occurrence is independently selected from halogen, C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, -$NR_{220}R_{225}$, OH, C≡N, -CO-(C_1-C_4 alkyl), -SO_2-$NR_{235}R_{240}$, -CO-$NR_{235}R_{240}$, -SO_2-(C_1-C_4 alkyl), =O, or

C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl or C_3-C_7 cycloalkyl, each of which is optionally substituted with 1, 2, or 3 R_{205} groups;

R_{215} at each occurrence is independently selected from C_1-C_6 alkyl, -$(CH_2)_{0-2}$-(aryl), C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, and -$(CH_2)_{0-2}$-(heteroaryl), -$(CH_2)_{0-2}$-(heterocyclyl), wherein

the aryl group at each occurrence is optionally substituted with 1, 2, or 3 groups that are independently R_{205} or R_{210}, and wherein

the heterocyclyl and heteroaryl groups at each occurrence are optionally substituted with 1, 2, or 3 R_{210};

R_{220} and R_{225} at each occurrence are independently selected from -H, -C_3-C_7 cycloalkyl, -(C_1-C_2 alkyl)-(C_3-C_7 cycloalkyl), -(C_1-C_6 alkyl)-O-(C_1-C_3 alkyl), -C_2-C_6 alkenyl, -C_2-C_6 alkynyl, -C_1-C_6 alkyl chain with one double bond and one triple bond, -aryl, -heteroaryl, and -heterocyclyl, or

-C_1-C_{10} alkyl optionally substituted with -OH, -NH_2 or halogen, wherein

the aryl, heterocyclyl and heteroaryl groups at each occurrence are optionally substituted with 1, 2, or 3 R_{270} groups

R_{235} and R_{240} at each occurrence are independently H, or C_1-C_6 alkyl;

R_{245} and R_{250} at each occurrence are independently selected from -H, C_1-C_4 alkyl, C_1-C_4 alkylaryl, C_1-C_4 alkylheteroaryl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkoxy, C_1-C_4 haloalkoxy, -$(CH_2)_{0-4}$-C_3-C_7 cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and phenyl; or

R_{245} and R_{250} are taken together with the carbon to which they are attached to form a carbocycle of 3, 4, 5, 6, or 7 carbon atoms, where one carbon atom is optionally replaced by a heteroatom selected from -O-, -S-, -SO_2-, and -NR_{220}-;

R_{255} and R_{260} at each occurrence are independently selected from -H, -$(CH_2)_{1-2}$-$S(O)_{0-2}$-(C_1-C_6 alkyl), -(C_1-C_4 alkyl)-aryl, -(C_1-C_4 alkyl)-heteroaryl, -(C_1-C_4 alkyl)-heterocyclyl, -aryl, -heteroaryl, -heterocyclyl, -$(CH_2)_{1-4}$-R_{265}-$(CH_2)_{0-4}$-aryl, -$(CH_2)_{1-4}$-R_{265}-$(CH_2)_{0-4}$-heteroaryl, -$(CH_2)_{1-4}$-R_{265}-$(CH_2)_{0-4}$-heterocyclyl, or

C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl or -$(CH_2)_{0-4}$-C_3-C_7 cycloalkyl, each of which is optionally substituted with 1, 2, or 3 R_{205} groups, wherein

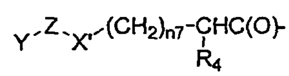
each aryl or phenyl is optionally substituted with 1, 2, or 3 groups that are independently R_{205}, R_{210}, or C_1-C_6 alkyl substituted with 1, 2, or 3 groups that are independently R_{205} or R_{210}, and wherein

each heterocyclyl is optionally substituted with 1, 2, 3, or 4 R_{210};

R_{265} at each occurrence is independently -O-, -S- or -N(C_1-C_6 alkyl)-;

R_{270} at each occurrence is independently R_{205}, halogen C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, $NR_{235}R_{240}$, -OH, -C≡N, -CO-(C_1-C_4 alkyl), -SO_2-$NR_{235}R_{240}$, -CO-$NR_{235}R_{240}$, -SO_2-(C_1-C_4 alkyl), =O, or

C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl or -$(CH_2)_{0-4}$-C_3-C_7 cycloalkyl, each of which is optionally substituted with 1, 2, or 3 R_{205} groups;

R_n is R'_{100}, -$SO_2R'_{100}$, -$(CRR')_{1-6}R'_{100}$, -C(=O)-$(CRR')_{0-6}R_{100}$, -C(=O)-$(CRR')_{1-6}$-O-R'_{100}, -C(=O)-$(CRR')_{1-6}$-S-R'_{100}, -C(=O)-$(CRR')_{1-6}$-C(=O)-R_{100}, -C(=O)-$(CRR')_{1-6}$-SO_2-R_{100}; -C(=O)-$(CRR')_{1-6}$-NR_{100}-R'_{100}; or  ;

R_4 is selected from the group consisting of H; NH_2; -NH-$(CH_2)_{n6}$-R_{4-1}; -NHR_8; -$NR_{50}C(O)R_5$; C_1-C_4 alkyl-NHC(O)R_5; -$(CH_2)_{0-4}R_8$; -O-C_1-C_4 alkanoyl; OH; C_6-C_{10} aryloxy optionally substituted with 1, 2, or 3 groups that are independently halogen, C_1-C_4 alkyl, -CO_2H, -C(O)-C_1-C_4 alkoxy, or C_1-C_4 alkoxy; C_1-C_6 alkoxy; aryl C_1-C_4 alkoxy; -$NR_{50}CO_2R_{51}$; -C_1-C_4 alkyl-$NR_{50}CO_2R_{51}$; -C≡N; -CF_3; -CF_2-CF_3; -C≡CH; -CH_2-CH=CH_2; -$(CH_2)_{1-4}$-R_{4-1}; -$(CH_2)_{1-4}$-NH-R_{4-1}; -O-$(CH_2)_{n6}$-R_{4-1}; -S-$(CH_2)_{n6}$-R_{4-1}; -$(CH_2)_{0-4}$-NHC(O)-$(CH_2)_{0-6}$-R_{52}; -$(CH_2)_{0-4}$-R_{53}-$(CH_2)_{0-4}$-R_{54}; wherein

n_6 is 0, 1, 2, or 3;

n_7 is 0, 1, 2, or 3;

R_{4-1} is selected from the group consisting of -SO_2-(C_1-C_8 alkyl), -SO-(C_1-C_8 alkyl), -S-(C_1-C_8 alkyl), -S-CO-

(C_1-C_6 alkyl), -SO_2-$NR_{4-2}R_{4-3}$; -CO-C_1-C_2 alkyl; -CO-$NR_{4-3}R_{4-4}$;

R_{4-2} and R_{4-3} are independently H, C_1-C_3 alkyl, or C_3-C_6 cycloalkyl;

R_{4-4} is alkyl, arylalkyl, alkanoyl, or arylalkanoyl;

R_{4-6} is-H or C_1-C_6 alkyl;

R_5 is selected from the group consisting of C_3-C_7 cycloalkyl; C_1-C_6 alkyl optionally substituted with 1, 2, or 3 groups that are independently halogen, -NR_6R_7, C_1-C_4 alkoxy, C_5-C_6 heterocycloalkyl, C_5-C_6 heteroaryl, C_6-C_{10} aryl, C_3-C_7 cycloalkyl C_1-C_4 alkyl, -S-C_1-C_4 alkyl, -SO_2-C_1-C_4 alkyl, -CO_2H, -$CONR_6R_7$, -CO_2-C_1-C_4 alkyl, C_6-C_{10} aryloxy; heteroaryl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, C_1-C_4 haloalkyl, or OH; heterocycloalkyl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, or C_2-C_4 alkanoyl; aryl optionally substituted with 1, 2, 3, or 4 groups that are independently halogen, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, or C_1-C_4 haloalkyl; and -NR_6R_7; wherein

R_6 and R_7 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkanoyl, phenyl, -SO_2-C_1-C_4 alkyl, phenyl C_1-C_4 alkyl;

R_8 is selected from the group consisting of -SO_2-heteroaryl, -SO_2-aryl, -SO_2-heterocycloalkyl, -SO_2-C_1-C_{10} alkyl, -C(O)NHR_9, heterocycloalkyl, -S-C_1-C_6 alkyl, -S-C_2-C_4 alkanoyl, wherein

R_9 is aryl C_1-C_4 alkyl, C_1-C_6 alkyl, or H;

R_{50} is H or C_1-C_6 alkyl;

R_{51} is selected from the group consisting of aryl C_1-C_4 alkyl; C_1-C_6 alkyl optionally substituted with 1, 2, or 3 groups that are independently halogen, cyano,

heteroaryl, $-NR_6R_7$, $-C(O)NR_6R_7$, C_3-C_7 cycloalkyl, or $-C_1$-C_4 alkoxy; heterocycloalkyl optionally substituted with 1 or 2 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, C_2-C_4 alkanoyl, aryl C_1-C_4 alkyl, and $-SO_2$ C_1-C_4 alkyl; alkenyl; alkynyl; heteroaryl optionally substituted with 1, 2, or 3 groups that are independently OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, NH_2, $NH(C_1$-C_6 alkyl) or $N(C_1$-C_6 alkyl)$(C_1$-C_6 alkyl); heteroarylalkyl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, NH_2, $NH(C_1$-C_6 alkyl) or $N(C_1$-C_6 alkyl)$(C_1$-C_6 alkyl); aryl; heterocycloalkyl; C_3-C_8 cycloalkyl; and cycloalkylalkyl; wherein the aryl; heterocycloalkyl, C_3-C_8 cycloalkyl, and cycloalkylalkyl groups are optionally substituted with 1, 2, 3, 4 or 5 groups that are independently halogen, CN, NO_2, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_6 alkanoyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, hydroxy, C_1-C_6 hydroxyalkyl, C_1-C_6 alkoxy C_1-C_6 alkyl, C_1-C_6 thioalkoxy, C_1-C_6 thioalkoxy C_1-C_6 alkyl, or C_1-C_6 alkoxy C_1-C_6 alkoxy;

R_{52} is heterocycloalkyl, heteroaryl, aryl, cycloalkyl, $-S(O)_{0-2}$-C_1-C_6 alkyl, CO_2H, $-C(O)NH_2$, -C(O)NH(alkyl), -C(O)N(alkyl)(alkyl), $-CO_2$-alkyl, $-NHS(O)_{0-2}$-C_1-C_6 alkyl, -N(alkyl)$S(O)_{0-2}$-C_1-C_6 alkyl, $-S(O)_{0-2}$-heteroaryl, $-S(O)_{0-2}$-aryl, -NH(arylalkyl), -N(alkyl)(arylalkyl), thioalkoxy, or alkoxy, each of which is optionally substituted with 1, 2, 3, 4, or 5 groups that are independently alkyl, alkoxy, thioalkoxy, halogen, haloalkyl, haloalkoxy, alkanoyl, NO_2, CN, alkoxycarbonyl, or aminocarbonyl;

R_{53} is absent, -O-, -C(O)-, -NH-, -N(alkyl)-, $-NH-S(O)_{0-2}-$, $-N(alkyl)-S(O)_{0-2}-$, $-S(O)_{0-2}-NH-$, $-S(O)_{0-2}-$ N(alkyl)-, -NH-C(S)-, or -N(alkyl)-C(S)-;

R_{54} is heteroaryl, aryl, arylalkyl, heterocycloalkyl, CO_2H, $-CO_2$-alkyl, -C(O)NH(alkyl), -C(O)N(alkyl)(alkyl), $-C(O)NH_2$, C_1-C_8 alkyl, OH, aryloxy, alkoxy, arylalkoxy, NH_2, NH(alkyl), N(alkyl) (alkyl), or $-C_1$-C_6 alkyl-CO_2-C_1-C_6 alkyl, each of which is optionally substituted with 1, 2, 3, 4, or 5 groups that are independently alkyl, alkoxy, CO_2H, $-CO_2$-alkyl, thioalkoxy, halogen, haloalkyl, haloalkoxy, hydroxyalkyl, alkanoyl, NO_2, CN, alkoxycarbonyl, or aminocarbonyl;

X' is selected from the group consisting of $-C_1$-C_6 alkylidenyl optionally optionally substituted with 1, 2, or 3 methyl groups; and $-NR_{4-6}-$; or

R_4 and R_{4-6} combine to form $-(CH_2)_{n10}-$, wherein

n_{10} is 1, 2, 3, or 4;

Z is selected from the group consisting of a bond; SO_2; SO; S; and C(O);

Y is selected from the group consisting of H; C_1-C_4 haloalkyl; C_5-C_6 heterocycloalkyl; C_6-C_{10} aryl; OH; $-N(Y_1)(Y_2)$; C_1-C_{10} alkyl optionally substituted with 1 thru 3 substituents which can be the same or different and are selected from the group consisting of halogen, hydroxy, alkoxy, thioalkoxy, and haloalkoxy; C_3-C_8 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from C_1-C_3 alkyl, and halogen; alkoxy; aryl optionally substituted with halogen, alkyl, alkoxy, CN or NO_2; arylalkyl optionally substituted with halogen, alkyl, alkoxy, CN or NO_2; wherein

Y_1 and Y_2 are the same or different and are H; C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 substituents selected from the group consisting of halogen, C_1-C_4 alkoxy, C_3-C_8 cycloalkyl, and OH; C_2-C_6 alkenyl; C_2-C_6 alkanoyl; phenyl; $-SO_2$-C_1-C_4 alkyl; phenyl C_1-C_4 alkyl; or C_3-C_8 cycloalkyl C_1-C_4 alkyl; or

Y_1, Y_2 and the nitrogen to which they are attached form a ring selected from the group consisting of piperazinyl, piperidinyl, morpholinyl, and pyrolidinyl, wherein each ring is optionally substituted with 1, 2, 3, or 4 groups that are independently C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 alkoxy C_1-C_6 alkyl, or halogen;

R_{100} and R'_{100} independently represent aryl, heteroaryl, -aryl-W-aryl, -aryl-W-heteroaryl, -aryl-W-heterocyclyl, -heteroaryl-W-aryl, -heteroaryl-W-heteroaryl, -heteroaryl-W- heterocyclyl, -heterocyclyl-W-aryl, -heterocyclyl-W-heteroaryl, -heterocyclyl-W-heterocyclyl, $-CH[(CH_2)_{0-2}-O-R_{150}]-(CH_2)_{0-2}$-aryl, $-CH[(CH_2)_{0-2}-O-R_{150}]-(CH_2)_{0-2}$-heterocyclyl or $-CH[(CH_2)_{0-2}-O-R_{150}]-(CH_2)_{0-2}$-heteroaryl, where the ring portions of each are optionally substituted with 1, 2, or 3 groups independently selected from

-OR, $-NO_2$, halogen, -C≡N, $-OCF_3$, $-CF_3$, $-(CH_2)_{0-4}-O-P(=O)(OR)(OR')$, $-(CH_2)_{0-4}-CO-NR_{105}R'_{105}$, $-(CH_2)_{0-4}-O-(CH_2)_{0-4}-CONR_{102}R_{102}'$, $-(CH_2)_{0-4}-CO-(C_1-C_{12}$ alkyl), $-(CH_2)_{0-4}-CO-(C_2-C_{12}$ alkenyl), $-(CH_2)_{0-4}-CO-(C_2-C_{12}$ alkynyl), $-(CH_2)_{0-4}-CO-(CH_2)_{0-4}(C_3-C_7$ cycloalkyl), $-(CH_2)_{0-4}-R_{110}$, $-(CH_2)_{0-4}-R_{120}$, $-(CH_2)_{0-4}-R_{130}$, $-(CH_2)_{0-4}-CO-R_{110}$, $-(CH_2)_{0-4}-CO-R_{120}$, $-(CH_2)_{0-4}-CO-R_{130}$, $-(CH_2)_{0-4}-CO-R_{140}$, $-(CH_2)_{0-4}-CO-O-R_{150}$, $-(CH_2)_{0-4}-SO_2-NR_{105}R'_{105}$, $-(CH_2)_{0-4}-SO-(C_1-C_8$ alkyl), $-(CH_2)_{0-4}-SO_2-(C_1-C_{12}$ alkyl), $-(CH_2)_{0-4}-SO_2-(CH_2)_{0-4}-(C_3-C_7$ cycloalkyl), $-(CH_2)_{0-4}-N(R_{150})-CO-O-R_{150}$, $-(CH_2)_{0-4}-N(R_{150})-CO-N(R_{150})_2$, $-(CH_2)_{0-4}-N(R_{150})-CS-N(R_{150})_2$, $-(CH_2)_{0-4}-N(R_{150})-CO-R_{105}$, $-(CH_2)_{0-4}-NR_{105}R'_{105}$, $-(CH_2)_{0-4}-R_{140}$, $-(CH_2)_{0-4}-O-CO-(C_1-C_6$ alkyl), $-(CH_2)_{0-4}-O-P(O)-(O-R_{110})_2$, $-(CH_2)_{0-4}-O-CO-N(R_{150})_2$, $-(CH_2)_{0-4}-O-CS-N(R_{150})_2$, $-(CH_2)_{0-4}-O-(R_{150})$, $-(CH_2)_{0-4}-O-R_{150}'-COOH$, $-(CH_2)_{0-4}-S-(R_{150})$, $-(CH_2)_{0-4}-N(R_{150})-SO_2-R_{105}$, $-(CH_2)_{0-4}-C_3-C_7$ cycloalkyl, (C_2-C_{10})alkenyl, or (C_2-C_{10})alkynyl, or

R_{100} is C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 R_{115} groups, or

R_{100} is -(C_1-C_6 alkyl)-O-C_1-C_6 alkyl) or -(C_1-C_6 alkyl)-S-(C_1-C_6 alkyl), each of which is optionally substituted with 1, 2, or 3 R_{115} groups, or

R_{100} is C_3-C_8 cycloalkyl optionally substituted with 1, 2, or 3 R_{115} groups;

W is -$(CH_2)_{0-4}$-, -O-, -S$(O)_{0-2}$-, -N(R_{135})-, -CR(OH)- or -C(O)-;

R_{102} and R_{102}' independently are hydrogen, or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 groups that are independently halogen, aryl or -R_{110};

R_{105} and R'_{105} independently represent -H, -R_{110}, -R_{120}, C_3-C_7 cycloalkyl, -(C_1-C_2 alkyl)-(C_3-C_7 cycloalkyl), -(C_1-C_6 alkyl)-O-(C_1-C_3 alkyl), C_2-C_6 alkenyl, C_2-C_6 alkynyl, or C_1-C_6 alkyl chain with one double bond and one triple bond, or

C_1-C_6 alkyl optionally substituted with -OH or -NH_2; or,

C_1-C_6 alkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, or

R_{105} and R'_{105} together with the atom to which they are attached form a 3 to 7 membered carbocylic ring, where one member is optionally a heteratom selected from -O-, -S$(O)_{0-2}$-, -N(R_{135})-, the ring being optionally substituted with 1, 2 or three R_{140} groups;

R_{115} at each occurrence is independently halogen, -OH, -CO_2R_{102}, -C_1-C_6 thioalkoxy, -CO_2-phenyl, -$NR_{105}R'_{135}$, -SO_2-(C_1-C_8 alkyl), -C(=O)R_{180}, R_{180}, -$CONR_{105}R'_{105}$, -$SO_2NR_{105}R'_{105}$, -NH-CO-(C_1-C_6 alkyl), -NH-C(=O)-OH, -NH-C(=O)-OR, -NH-C(=O)-O-phenyl, -O-C(=O)-(C_1-C_6 alkyl), -O-C(=O)-amino, -O-C(=O)-mono- or dialkylamino, -O-C(=O)-phenyl, -O-(C_1-C_6 alkyl)-CO_2H, -NH-SO_2-(C_1-C_6 alkyl), C_1-C_6 alkoxy or C_1-C_6 haloalkoxy;

R_{135} is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, -$(CH_2)_{0-2}$-(aryl), -$(CH_2)_{0-2}$-(heteroaryl), or -$(CH_2)_{0-2}$-(heterocyclyl);

R_{140} is heterocyclyl optionally substituted with 1, 2, 3, or 4 groups independently selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, hydroxy, cyano, nitro, amino, mono(C_1-C_6)alkylamino, di(C_1-C_6)alkylamino, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, amino(C_1-C_6)alkyl, mono(C_1-C_6)alkylamino(C_1-C_6)alkyl, di(C_1-C_6)alkylamino(C_1-C_6)alkyl, and =O;

R_{150} is hydrogen, C_3-C_7 cycloalkyl, -(C_1-C_2 alkyl)-(C_3-C_7 cycloalkyl), C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkyl with one double bond and one triple bond, -R_{110}, -R_{120}, or

C_1-C_6 alkyl optionally substituted with 1, 2, 3, or 4 groups independently selected from -OH, -NH_2, C_1-C_3 alkoxy, R_{110}, and halogen;

R_{150}' is C_3-C_7 cycloalkyl, -(C_1-C_3 alkyl)-(C_3-C_7 cycloalkyl), C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkyl with one double bond and one triple bond, -R_{110}, -R_{120}, or

C_1-C_6 alkyl optionally substituted with 1, 2, 3, or 4 groups independently selected from -OH, -NH_2, C_1-C_3 alkoxy, R_{110}, and halogen;

R_{180} is selected from morpholinyl, thiomorpholinyl, piperazinyl, piperidinyl, homomorpholinyl, homothiomorpholinyl, homothiomorpholinyl S-oxide, homothiomorpholinyl S,S-dioxide, pyrrolinyl and pyrrolidinyl, each of which is optionally substituted with 1, 2, 3, or 4 groups independently selected from C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, hydroxy, cyano, nitro, amino, mono(C_1-C_6)alkylamino, di(C_1-C_6)alkylamino, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, amino(C_1-C_6)alkyl, mono(C_1-C_6)alkylamino(C_1-C_6)alkyl, di(C_1-C_6)alkylamino(C_1-C_6)alkyl, and =O;

R_{110} is aryl optionally substituted with 1 or 2 R_{125} groups;

R_{125} at each occurrence is independently halogen, amino, mono- or dialkylamino, -OH, -C≡N, $-SO_2-NH_2$, $-SO_2-NH-C_1-C_6$ alkyl, $-SO_2-N(C_1-C_6$ alkyl$)_2$, $-SO_2-(C_1-C_4$ alkyl), $-CO-NH_2$, $-CO-NH-C_1-C_6$ alkyl, or $-CO-N(C_1-C_6$ alkyl$)_2$, or

C_1-C_6 alkyl, C_2-C_6 alkenyl or C_2-C_6 alkynyl, each of which is optionally substituted with 1, 2, or 3 groups that are independently selected from C_1-C_3 alkyl, halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, amino, and mono- and dialkylamino, or

C_1-C_6 alkoxy optionally substituted with one, two or three of halogen;

R_{120} is heteroaryl, which is optionally substituted with 1 or 2 R_{125} groups; and

R_{130} is heterocyclyl optionally substituted with 1 or 2 R_{125} groups; and

R_2 is selected from the group consisting of H; C_1-C_6 alkyl, optionally substituted with 1, 2, or 3 substituents that are independently selected from the group consisting of C_1-C_3 alkyl, halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, and $-NR_{1-a}R_{1-b}$; wherein

R_{1-a} and R_{1-b} are -H or C_1-C_6 alkyl;

$-(CH_2)_{0-4}$-aryl; $-(CH_2)_{0-4}$-heteroaryl; C_2-C_6 alkenyl; C_2-C_6 alkynyl; $-CONR_{N-2}R_{N-3}$; $-SO_2NR_{N-2}R_{N-3}$; $-CO_2H$; and $-CO_2-(C_1-C_4$ alkyl);

R_3 is selected from the group consisting of H; C_1-C_6 alkyl, optionally substituted with 1, 2, or 3 substituents independently selected from the group consisting of C_1-C_3 alkyl, halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, and $-NR_{1-a}R_{1-b}$; $-(CH_2)_{0-4}$-aryl; $-(CH_2)_{0-4}$-heteroaryl; C_2-C_6 alkenyl; C_2-C_6 alkynyl; $-CO-NR_{N-2}R_{N-3}$; $-SO_2-NR_{N-2}R_{N-3}$; $-CO_2H$; and $-CO-O-(C_1-C_4$ alkyl);

wherein

R_{N-2} and R_{N-3} at each occurrence are independently selected from the group consisting of

$-C_1-C_8$ alkyl optionally substituted with 1, 2, or 3 groups independently selected from the group consisting of -OH, $-NH_2$, phenyl and halogen; $-C_3-C_8$ cycloalkyl; $-(C_1-C_2$ alkyl$)-(C_3-C_8$ cycloalkyl); $-(C_1-C_6$ alkyl$)-O-(C_1-C_3$ alkyl); $-C_2-C_6$ alkenyl; $-C_2-C_6$ alkynyl; $-C_1-C_6$ alkyl chain with one double bond and one triple bond; aryl; heteroaryl; heterocycloalkyl; or

R_{N-2}, R_{N-3} and the nitrogen to which they are attached form a 5, 6, or 7 membered heterocycloalkyl or heteroaryl group, wherein said heterocycloalkyl or heteroaryl group is optionally fused to a benzene, pyridine, or pyrimidine ring, and said groups are unsubstituted or substituted with 1, 2, 3, 4, or 5 groups that at each occurrence are independently C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, halo C_1-C_6 alkyl, halo C_1-C_6 alkoxy, -CN, $-NO_2$, $-NH_2$, $NH(C_1-C_6$ alkyl), $N(C_1-C_6$ alkyl$)(C_1-C_6$ alkyl), -OH, $-C(O)NH_2$, $-C(O)NH(C_1-C_6$ alkyl), $-C(O)N(C_1-C_6$ alkyl$)(C_1-C_6$ alkyl), C_1-C_6 alkoxy C_1-C_6 alkyl, C_1-C_6 thioalkoxy, and C_1-C_6 thioalkoxy C_1-C_6 alkyl;

or wherein,

R_2, R_3 and the carbon to which they are attached form a carbocycle of three thru seven carbon atoms, wherein one carbon atom is optionally replaced by a group selected from-O-, -S-, $-SO_2-$, or $-NR_{N-2}-$.

The invention also provides a method for making a compound of formula (I)

or a pharmaceutically acceptable salt or ester thereof.

The invention also includes a method of treating a patient who has, or in preventing a patient from getting, a disease or condition selected from the group consisting of Alzheimer's disease, for helping prevent or delay the onset of Alzheimer's disease, for treating patients with mild cognitive

impairment (MCI) and preventing or delaying the onset of Alzheimer's disease in those who would progress from MCI to AD, for treating Down's syndrome, for treating humans who have Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-Type, for treating cerebral amyloid angiopathy and preventing its potential consequences, i.e. single and recurrent lobar hemorrhages, for treating other degenerative dementias, including dementias of mixed vascular and degenerative origin, dementia associated with Parkinson's disease, dementia associated with progressive supranuclear palsy, dementia associated with cortical basal degeneration, or diffuse Lewy body type of Alzheimer's disease and who is in need of such treatment which comprises administration of a therapeutically effective amount of a compound of formula (I).

The invention also includes methods for inhibiting beta-secretase activity, for inhibiting cleavage of amyloid precursor protein (APP), in a reaction mixture, at a site between Met596 and Asp597, numbered for the APP-695 amino acid isotype; or at a corresponding site of an isotype or mutant thereof, for inhibiting production of amyloid beta peptide (A beta) in a cell, for inhibiting the production of beta-amyloid plaque in an animal, and for treating or preventing a disease characterized by beta-amyloid deposits in the brain which comprise administration of a therapeutically effective amount of a compound of formula (I).

The invention also includes a pharmaceutical composition that comprises a compound of formula (I), or pharmaceutically acceptable salts and esters thereof, and one or more pharmaceutically acceptable inert carriers.

The invention also includes the use of compounds of formula (I), or pharmaceutically acceptable salts and esters thereof, for the manufacture of a medicament for use in treating a patient who has, or in preventing a patient from getting, a disease or condition selected from the group

consisting of Alzheimer's disease, for helping prevent or delay the onset of Alzheimer's disease, for treating patients with mild cognitive impairment (MCI) and preventing or delaying the onset of Alzheimer's disease in those who would progress from MCI to AD, for treating Down's syndrome, for treating humans who have Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-Type, for treating cerebral amyloid angiopathy and preventing its potential consequences, i.e. single and recurrent lobar hemorrhages, for treating other degenerative dementias, including dementias of mixed vascular and degenerative origin, dementia associated with Parkinson's disease, dementia associated with progressive supranuclear palsy, dementia associated with cortical basal degeneration, diffuse Lewy body type of Alzheimer's disease and who is in need of such treatment.

The invention provides compounds, compositions, kits, and methods for inhibiting beta-secretase-mediated cleavage of amyloid precursor protein (APP). More particularly, the compounds, compositions, and methods of the invention are effective to inhibit the production of A beta peptide and to treat or prevent any human or veterinary disease or condition associated with a pathological form of A beta peptide.

The compounds, compositions, and methods of the invention are useful for treating humans who have Alzheimer's Disease (AD), for helping prevent or delay the onset of AD, for treating patients with mild cognitive impairment (MCI), and preventing or delaying the onset of AD in those patients who would otherwise be expected to progress from MCI to AD, for treating Down's syndrome, for treating Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch Type, for treating cerebral beta-amyloid angiopathy and preventing its potential consequences such as single and recurrent lobar hemorrhages, for treating other degenerative dementias, including dementias of mixed vascular and degenerative origin, for treating

dementia associated with Parkinson's disease, dementia associated with progressive supranuclear palsy, dementia associated with cortical basal degeneration, and diffuse Lewy body type AD.

The invention also provides intermediates and methods useful for preparing the compounds of Formula I, or pharmaceutically acceptable salts or esters thereof.

The compounds of the invention possess beta-secretase inhibitory activity. The inhibitory activities of the compounds of the invention are readily demonstrated, for example, using one or more of the assays described herein or known in the art.

Unless the substituents for a particular formula are expressly defined for that formula, they are understood to carry the definitions set forth in connection with the preceeding formula to which the particular formula makes reference.

DETAILED DESCRIPTION OF THE INVENTION

Preferred compounds of formula I include compounds of formula I-1, i.e., compounds of formula I wherein Rn is $-C(=O)-(CRR')_{1-6}R_{100}$, and wherein R, R' and R_{100} are as defined above.

Preferred compounds of formula I-1 include compounds wherein Rn is $-C(=O)-(CRR')_{0-6}R_{100}$, where R and R' are as defined above and where R_{100} is as defined above, but is not -heterocyclyl-W-aryl.

Preferred compounds of formula I-1 also include those wherein Rn is $-C(=O)-R_{100}$; and

R_{100} represents aryl, or heteroaryl, where the ring portions of each are optionally substituted with 1, 2, or 3 groups independently selected from

$-OR$, $-NO_2$, C_1-C_6 alkyl, halogen, $-C\equiv N$, $-OCF_3$, $-CF_3$, $-(CH_2)_{0-4}-O-P(=O)(OR)(OR')$, $-(CH_2)_{0-4}-CO-NR_{105}R'_{105}$, $-(CH_2)_{0-4}-O-(CH_2)_{0-4}-CONR_{102}R_{102}'$, $-(CH_2)_{0-4}-CO-(C_1-C_{12}\text{ alkyl})$, $-(CH_2)_{0-}$

$_4$-CO-(C_2-C_{12} alkenyl), -$(CH_2)_{0-4}$-CO-(C_2-C_{12} alkynyl), -$(CH_2)_{0-4}$-CO-$(CH_2)_{0-4}$(C_3-C_7 cycloalkyl), -$(CH_2)_{0-4}$-R_{110}, -$(CH_2)_{0-4}$-R_{120}, -$(CH_2)_{0-4}$-R_{130}, -$(CH_2)_{0-4}$-CO-R_{110}, -$(CH_2)_{0-4}$-CO-R_{120}, -$(CH_2)_{0-4}$-CO-R_{130}, -$(CH_2)_{0-4}$-CO-R_{140}, -$(CH_2)_{0-4}$-CO-O-R_{150}, -$(CH_2)_{0-4}$-SO_2-$NR_{105}R'_{105}$, -$(CH_2)_{0-4}$-SO-(C_1-C_8 alkyl), -$(CH_2)_{0-4}$-SO_2-(C_1-C_{12} alkyl), -$(CH_2)_{0-4}$-SO_2-$(CH_2)_{0-4}$-(C_3-C_7 cycloalkyl), -$(CH_2)_{0-4}$-N(R_{150})-CO-O-R_{150}, -$(CH_2)_{0-4}$-N(R_{150})-CO-N$(R_{150})_2$, -$(CH_2)_{0-4}$-N(R_{150})-CS-N$(R_{150})_2$, -$(CH_2)_{0-4}$-N(R_{150})-CO-R_{105}, -$(CH_2)_{0-4}$-$NR_{105}R'_{105}$, -$(CH_2)_{0-4}$-R_{140}, -$(CH_2)_{0-4}$-O-CO-(C_1-C_6 alkyl), -$(CH_2)_{0-4}$-O-P(O)-$(O-R_{110})_2$, -$(CH_2)_{0-4}$-O-CO-N$(R_{150})_2$, -$(CH_2)_{0-4}$-O-CS-N$(R_{150})_2$, -$(CH_2)_{0-4}$-O-(R_{150}), -$(CH_2)_{0-4}$-O-R_{150}'-COOH, -$(CH_2)_{0-4}$-S-(R_{150}), -$(CH_2)_{0-4}$-N(R_{150})-SO_2-R_{105}, -$(CH_2)_{0-4}$-C_3-C_7 cycloalkyl, (C_2-C_{10})alkenyl, or (C_2-C_{10})alkynyl.

Preferred compounds of formula I-1 further include those wherein Rn is -C(=O)-aryl or -C(=O)-heteroaryl wherein the ring portions of each are optionally substituted with 1, 2, or 3 groups independently selected from:

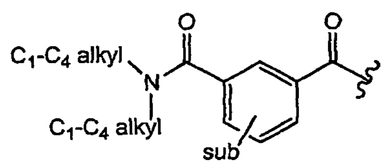
-OR, -NO_2, C_1-C_6 alkyl, halogen, -C≡N, -OCF_3, -CF_3, -$(CH_2)_{0-4}$-CO-$NR_{105}R'_{105}$, -$(CH_2)_{0-4}$-O-$(CH_2)_{0-4}$-$CONR_{102}R_{102}'$, -$(CH_2)_{0-4}$-CO-(C_1-C_{12} alkyl), -$(CH_2)_{0-4}$-CO-(C_2-C_{12} alkenyl), -$(CH_2)_{0-4}$-CO-(C_2-C_{12} alkynyl), -$(CH_2)_{0-4}$-R_{110}, -$(CH_2)_{0-4}$-R_{120}, -$(CH_2)_{0-4}$-R_{130}, -$(CH_2)_{0-4}$-CO-R_{110}, -$(CH_2)_{0-4}$-CO-R_{120}, -$(CH_2)_{0-4}$-CO-R_{130}, -$(CH_2)_{0-4}$-CO-R_{140}, -$(CH_2)_{0-4}$-CO-O-R_{150}, -$(CH_2)_{0-4}$-SO_2-$NR_{105}R'_{105}$, -$(CH_2)_{0-4}$-SO-(C_1-C_8 alkyl), -$(CH_2)_{0-4}$-SO_2-(C_1-C_{12} alkyl), -$(CH_2)_{0-4}$-N(R_{150})-CO-O-R_{150}, -$(CH_2)_{0-4}$-N(R_{150})-CO-N$(R_{150})_2$, -$(CH_2)_{0-4}$-N(R_{150})-CO-R_{105}, -$(CH_2)_{0-4}$-$NR_{105}R'_{105}$, -$(CH_2)_{0-4}$-R_{140}, -$(CH_2)_{0-4}$-O-CO-(C_1-C_6 alkyl), -$(CH_2)_{0-4}$-O-CO-N$(R_{150})_2$, -$(CH_2)_{0-4}$-O-(R_{150}), -$(CH_2)_{0-4}$-N(R_{150})-SO_2-R_{105}, -$(CH_2)_{0-4}$- C_3-C_7 cycloalkyl, (C_2-C_{10})alkenyl, or (C_2-C_{10})alkynyl.

Preferred compounds of formula I-1 further include those wherein Rn is -C(=O)-aryl or -C(=O)-heteroaryl, wherein the

ring portions of each are optionally substituted with 1 or 2 groups independently selected from:

C_1-C_6 alkyl, halogen, $-(CH_2)_{0-4}$-CO-$NR_{105}R'_{105}$, $-(CH_2)_{0-4}$-O-CO-$N(R_{150})_2$, $-(CH_2)_{0-4}$-$N(R_{150})$-SO_2-R_{105}, $-(CH_2)_{0-4}$-SO_2-$NR_{105}R'_{105}$, C_3-C_7 cycloalkyl, $(C_2$-$C_{10})$alkenyl, $-(CH_2)_{0-4}$-R_{110}, $-(CH_2)_{0-4}$-R_{120}, $-(CH_2)_{0-4}$-R_{130}, or $(C_2$-$C_{10})$alkynyl.

Preferred compounds of formula I-1 further include those wherein Rn is:



wherein sub is hydrogen or is C_1-C_6 alkyl, halogen, $-(CH_2)_{0-4}$-CO-$NR_{105}R'_{105}$, $-(CH_2)_{0-4}$-O-CO-$N(R_{150})_2$, $-(CH_2)_{0-4}$-$N(R_{150})$-SO_2-R_{105}, $-(CH_2)_{0-4}$-SO_2-$NR_{105}R'_{105}$, C_3-C_7 cycloalkyl, $-(C_2$-$C_{10})$alkenyl, $-(CH_2)_{0-4}$-R_{110}, $-(CH_2)_{0-4}$-R_{120}, $-(CH_2)_{0-4}$-R_{130}, or $(C_2$-$C_{10})$alkynyl.

Preferred compounds of formula I also include compounds of formula I-2, i.e., compounds of formula I wherein Rn is

$$Y^{\diagup}Z^{\diagdown}X'^{\diagup}(CH_2)_{n7}\text{-}\underset{\displaystyle R_4}{\underset{|}{C}}HC(O)\text{-}$$

wherein

R_4 is selected from the group consisting of H; NH_2; $-NH-(CH_2)_{n6}-R_{4-1}$; $-NHR_8$; $-NR_{50}C(O)R_5$; C_1-C_4 alkyl-NHC(O)R_5; $-(CH_2)_{0-4}R_8$; -O-C_1-C_4 alkanoyl; OH; C_6-C_{10} aryloxy optionally substituted with 1, 2, or 3 groups that are independently halogen, C_1-C_4 alkyl, $-CO_2H$, -C(O)-C_1-C_4 alkoxy, or C_1-C_4 alkoxy; C_1-C_6 alkoxy; aryl C_1-C_4 alkoxy; $-NR_{50}CO_2R_{51}$; $-C_1$-C_4 alkyl-$NR_{50}CO_2R_{51}$; $-C\equiv N$; $-CF_3$; $-CF_2-CF_3$; $-C\equiv CH$; $-CH_2-CH=CH_2$; $-(CH_2)_{1-4}-R_{4-1}$; $-(CH_2)_{1-4}-NH-R_{4-1}$; $-O-(CH_2)_{n6}-R_{4-1}$; $-S-(CH_2)_{n6}-R_{4-1}$; $-(CH_2)_{0-4}-NHC(O)-(CH_2)_{0-6}-R_{52}$; $-(CH_2)_{0-4}-R_{53}-(CH_2)_{0-4}-R_{54}$;

wherein

n_6 is 0, 1, 2, or 3;

n_7 is 0, 1, 2, or 3;

R_{4-1} is selected from the group consisting of $-SO_2-(C_1-C_8$ alkyl), $-SO-(C_1-C_8$ alkyl), $-S-(C_1-C_8$ alkyl), $-S-CO-(C_1-C_6$ alkyl), $-SO_2-NR_{4-2}R_{4-3}$; $-CO-C_1-C_2$ alkyl; $-CO-NR_{4-3}R_{4-4}$;

R_{4-2} and R_{4-3} are independently H, C_1-C_3 alkyl, or C_3-C_6 cycloalkyl;

R_{4-4} is alkyl, arylalkyl, alkanoyl, or arylalkanoyl;

R_{4-6} is–H or C_1-C_6 alkyl;

R_5 is selected from the group consisting of C_3-C_7 cycloalkyl; C_1-C_6 alkyl optionally substituted with 1, 2, or 3 groups that are independently halogen, $-NR_6R_7$, C_1-C_4 alkoxy, C_5-C_6 heterocycloalkyl, C_5-C_6 heteroaryl, C_6-C_{10} aryl, C_3-C_7 cycloalkyl C_1-C_4 alkyl, $-S-C_1-C_4$ alkyl, $-SO_2-C_1-C_4$ alkyl, $-CO_2H$, $-CONR_6R_7$, $-CO_2-C_1-C_4$ alkyl, C_6-C_{10} aryloxy; heteroaryl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, C_1-C_4 haloalkyl, or OH; heterocycloalkyl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, or C_2-C_4 alkanoyl; aryl optionally substituted with 1, 2, 3, or 4 groups that are independently halogen, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, or C_1-C_4 haloalkyl; and $-NR_6R_7$; wherein

R_6 and R_7 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkanoyl, phenyl, $-SO_2-C_1-C_4$ alkyl, phenyl C_1-C_4 alkyl;

R_8 is selected from the group consisting of $-SO_2$-heteroaryl, $-SO_2$-aryl, $-SO_2$-heterocycloalkyl, $-SO_2-C_1-C_{10}$ alkyl, $-C(O)NHR_9$, heterocycloalkyl, $-S-C_1-C_6$ alkyl, $-S-C_2-C_4$ alkanoyl, wherein

R_9 is aryl C_1-C_4 alkyl, C_1-C_6 alkyl, or H;

R_{50} is H or C_1-C_6 alkyl;

R_{51} is selected from the group consisting of aryl C_1-C_4 alkyl; C_1-C_6 alkyl optionally substituted with 1, 2, or 3 groups that are independently halogen, cyano, heteroaryl, -NR_6R_7, -C(O)NR_6R_7, C_3-C_7 cycloalkyl, or -C_1-C_4 alkoxy; heterocycloalkyl optionally substituted with 1 or 2 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, C_2-C_4 alkanoyl, aryl C_1-C_4 alkyl, and -SO_2 C_1-C_4 alkyl; alkenyl; alkynyl; heteroaryl optionally substituted with 1, 2, or 3 groups that are independently OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, NH_2, NH(C_1-C_6 alkyl) or N(C_1-C_6 alkyl)(C_1-C_6 alkyl); heteroarylalkyl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, NH_2, NH(C_1-C_6 alkyl) or N(C_1-C_6 alkyl)(C_1-C_6 alkyl); aryl; heterocycloalkyl; C_3-C_8 cycloalkyl; and cycloalkylalkyl; wherein the aryl; heterocycloalkyl, C_3-C_8 cycloalkyl, and cycloalkylalkyl groups are optionally substituted with 1, 2, 3, 4 or 5 groups that are independently halogen, CN, NO_2, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_6 alkanoyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, hydroxy, C_1-C_6 hydroxyalkyl, C_1-C_6 alkoxy C_1-C_6 alkyl, C_1-C_6 thioalkoxy, C_1-C_6 thioalkoxy C_1-C_6 alkyl, or C_1-C_6 alkoxy C_1-C_6 alkoxy;

R_{52} is heterocycloalkyl, heteroaryl, aryl, cycloalkyl, -S(O)$_{0-2}$-C_1-C_6 alkyl, CO_2H, -C(O)NH_2, -C(O)NH(alkyl), -C(O)N(alkyl)(alkyl), -CO_2-alkyl, -NHS(O)$_{0-2}$-C_1-C_6 alkyl, -N(alkyl)S(O)$_{0-2}$-C_1-C_6 alkyl, -S(O)$_{0-2}$-heteroaryl, -S(O)$_{0-2}$-aryl, -NH(arylalkyl), -N(alkyl)(arylalkyl), thioalkoxy, or alkoxy, each of which is optionally substituted with 1, 2, 3, 4, or 5 groups that are independently alkyl, alkoxy,

thioalkoxy, halogen, haloalkyl, haloalkoxy, alkanoyl, NO_2, CN, alkoxycarbonyl, or aminocarbonyl;

R_{53} is absent, -O-, -C(O)-, -NH-, -N(alkyl)-, -NH-S(O)$_{0-2}$-, -N(alkyl)-S(O)$_{0-2}$-, -S(O)$_{0-2}$-NH-, -S(O)$_{0-2}$- N(alkyl)-, -NH-C(S)-, or -N(alkyl)-C(S)-;

R_{54} is heteroaryl, aryl, arylalkyl, heterocycloalkyl, CO_2H, -CO_2-alkyl, -C(O)NH(alkyl), -C(O)N(alkyl) (alkyl), -C(O)NH_2, C_1-C_8 alkyl, OH, aryloxy, alkoxy, arylalkoxy, NH_2, NH(alkyl), N(alkyl) (alkyl), or -C_1-C_6 alkyl-CO_2-C_1-C_6 alkyl, each of which is optionally substituted with 1, 2, 3, 4, or 5 groups that are independently alkyl, alkoxy, CO_2H, -CO_2-alkyl, thioalkoxy, halogen, haloalkyl, haloalkoxy, hydroxyalkyl, alkanoyl, NO_2, CN, alkoxycarbonyl, or aminocarbonyl;

X' is selected from the group consisting of -C_1-C_6 alkylidenyl optionally optionally substituted with 1, 2, or 3 methyl groups; and -NR_{4-6}-; or

R_4 and R_{4-6} combine to form -$(CH_2)_{n10}$-, wherein n_{10} is 1, 2, 3, or 4;

Z is selected from the group consisting of a bond; SO_2; SO; S; and C(O);

Y is selected from the group consisting of H; C_1-C_4 haloalkyl; C_5-C_6 heterocycloalkyl; C_6-C_{10} aryl; OH; -N(Y_1)(Y_2); C_1-C_{10} alkyl optionally substituted with 1 thru 3 substituents which can be the same or different and are selected from the group consisting of halogen, hydroxy, alkoxy, thioalkoxy, and haloalkoxy; C_3-C_8 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from C_1-C_3 alkyl, and halogen; alkoxy; aryl optionally substituted with halogen, alkyl, alkoxy, CN or NO_2; arylalkyl optionally substituted with halogen, alkyl, alkoxy, CN or NO_2; wherein

Y_1 and Y_2 are the same or different and are H; C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 substituents selected from the group consisting of halogen, C_1-C_4 alkoxy, C_3-C_8 cycloalkyl, and OH; C_2-C_6 alkenyl; C_2-C_6 alkanoyl; phenyl; -SO_2-C_1-C_4 alkyl; phenyl C_1-C_4 alkyl; or C_3-C_8 cycloalkyl C_1-C_4 alkyl; or

Y_1, Y_2 and the nitrogen to which they are attached form a ring selected from the group consisting of piperazinyl, piperidinyl, morpholinyl, and pyrolidinyl, wherein each ring is optionally substituted with 1, 2, 3, or 4 groups that are independently C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 alkoxy C_1-C_6 alkyl, or halogen.

Preferred compounds of formula I-2 also include compounds wherein Rn is

$$Y\diagup^{Z}\diagdown X'-\underset{\underset{NH_2}{|}}{C}HC(O)-$$

wherein

X' is C_1-C_4 alkylidenyl optionally substituted with 1, 2, or 3 methyl groups; or -NR_{4-6}-, where R_{4-6} is-H or C_1-C_6 alkyl; or

R_4 and R_{4-6} combine to form -$(CH_2)_{n10}$-, where R_4 and R_{4-6} are as defined above, wherein

n_{10} is 1, 2, 3, or 4;

Z is selected from a bond; SO_2; SO; S; and C(O);

Y is selected from H; C_1-C_4 haloalkyl; C_5-C_6 heterocycloalkyl containing at least one N, O, or S; phenyl; OH; -N(Y_1)(Y_2); C_1-C_{10} alkyl optionally substituted with 1 thru 3 substituents which can be the same or different and are selected from halogen, hydroxy, alkoxy, thioalkoxy, and haloalkoxy; C_3-C_8 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from C_1-C_3 alkyl, and halogen; alkoxy; phenyl optionally substituted with halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN or NO_2; phenyl C_1-C_4 alkyl

optionally substituted with halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN or NO_2; wherein

Y_1 and Y_2 are the same or different and are H; C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 substituents selected from the group consisting of halogen, C_1-C_4 alkoxy, C_3-C_8 cycloalkyl, and OH; C_2-C_6 alkenyl; C_2-C_6 alkanoyl; phenyl; -SO_2-C_1-C_4 alkyl; phenyl C_1-C_4 alkyl; and C_3-C_8 cycloalkyl C_1-C_4 alkyl; or

-$N(Y_1)(Y_2)$ forms a ring selected from piperazinyl, piperidinyl, morpholinyl, and pyrolidinyl, wherein each ring is optionally substituted with 1, 2, 3, or 4 groups that are independently C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 alkoxy C_1-C_6 alkyl, or halogen.

Preferred compounds of formula I-2 further include compounds wherein

X' is C_1-C_4 alkylidenyl optionally substituted with 1, 2, or 3 methyl groups;

Z is selected from SO_2; SO; S; and C(O);

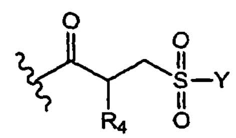
Y is selected from H; C_1-C_4 haloalkyl; C_5-C_6 heterocycloalkyl containing at least one N, O, or S; phenyl; OH; -$N(Y_1)(Y_2)$; C_1-C_{10} alkyl optionally substituted with 1 thru 3 substituents which can be the same or different and are selected from the group consisting of halogen, hydroxy, alkoxy, thioalkoxy, and haloalkoxy; C_3-C_8 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from C_1-C_3 alkyl, and halogen; alkoxy; phenyl optionally substituted with halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN or NO_2; phenyl C_1-C_4 alkyl optionally substituted with halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN or NO_2; wherein

Y_1 and Y_2 are the same or different and are H; C_1-C_6 alkyl optionally substituted with 1, 2, or 3 substituents selected from the group consisting of halogen, C_1-C_4 alkoxy, C_3-C_8 cycloalkyl, and OH; C_2-C_6 alkenyl; C_2-C_6

alkanoyl; phenyl; $-SO_2-C_1-C_4$ alkyl; phenyl C_1-C_4 alkyl; or C_3-C_8 cycloalkyl C_1-C_4 alkyl; or

$-N(Y_1)(Y_2)$ forms a ring selected from piperazinyl, piperidinyl, morpholinyl, and pyrolidinyl, wherein each ring is optionally substituted with 1, 2, 3, or 4 groups that are independently C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 alkoxy C_1-C_6 alkyl, or halogen.

Preferred compounds of formula I-2 include compounds wherein Rn is



and wherein R_4 is NH_2; $-NH-(CH_2)_{n6}-R_{4-1}$; $-NHR_8$; $-NR_{50}C(O)R_5$; or $-NR_{50}CO_2R_{51}$ wherein

n_6 is 0, 1, 2, or 3;

n_7 is 0, 1, 2, or 3;

R_{4-1} is selected from the group consisting of $-SO_2-(C_1-C_8$ alkyl), $-SO-(C_1-C_8$ alkyl), $-S-(C_1-C_8$ alkyl), $-S-CO-(C_1-C_6$ alkyl), $-SO_2-NR_{4-2}R_{4-3}$; $-CO-C_1-C_2$ alkyl; $-CO-NR_{4-3}R_{4-4}$;

R_{4-2} and R_{4-3} are independently H, C_1-C_3 alkyl, or C_3-C_6 cycloalkyl;

R_{4-4} is alkyl, phenylalkyl, C_2-C_4 alkanoyl, or phenylalkanoyl;

R_5 is cyclopropyl; cyclobutyl; cyclopentyl; or cyclohexyl; wherein each cycloalkyl group is optionally substituted with one or two groups that are C_1-C_6 alkyl, more preferably C_1-C_2 alkyl, C_1-C_6 alkoxy, more preferably C_1-C_2 alkoxy, CF_3, OH, NH_2, $NH(C_1-C_6$ alkyl), $N(C_1-C_6$ alkyl)$(C_1-C_6$ alkyl), halogen, CN, or NO_2; or the cycloalkyl group is substituted with 1 or 2 groups that are independently CF_3, Cl, F, methyl, ethyl or cyano; C_1-C_6 alkyl optionally substituted with 1, 2, or 3 groups that are independently

halogen, $-NR_6R_7$, C_1-C_4 alkoxy, C_5-C_6 heterocycloalkyl, C_5-C_6 heteroaryl, phenyl, C_3-C_7 cycloalkyl, -S-C_1-C_4 alkyl, $-SO_2$-C_1-C_4 alkyl, $-CO_2H$, $-CONR_6R_7$, $-CO_2$-C_1-C_4 alkyl, or phenyloxy; heteroaryl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, C_1-C_4 haloalkyl, or OH; heterocycloalkyl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, or C_2-C_4 alkanoyl; phenyl optionally substituted with 1, 2, 3, or 4 groups that are independently halogen, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, or C_1-C_4 haloalkyl; and $-NR_6R_7$; wherein

R_6 and R_7 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_6 alkanoyl, phenyl, $-SO_2$-C_1-C_4 alkyl, and phenyl C_1-C_4 alkyl;

R_8 is selected from the group consisting of $-SO_2$-heteroaryl optionally substituted with 1 or 2 groups that are independently C_1-C_4 alkyl or halogen;, $-SO_2$-aryl, $-SO_2$-heterocycloalkyl, $-C(O)NHR_9$, heterocycloalkyl, -S-C_2-C_4 alkanoyl, wherein

R_9 is phenyl C_1-C_4 alkyl, C_1-C_6 alkyl, or H;

R_{50} is H or C_1-C_6 alkyl; and

R_{51} is selected from the group consisting of phenyl C_1-C_4 alkyl; C_1-C_6 alkyl optionally substituted with 1, 2, or 3 groups that are independently halogen, cyano, -NR_6R_7, $-C(O)NR_6R_7$, C_3-C_7 or $-C_1$-C_4 alkoxy; heterocycloalkyl optionally substituted with 1 or 2 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, C_2-C_4 alkanoyl, phenyl C_1-C_4 alkyl, and $-SO_2$ C_1-C_4 alkyl; heterocycloalkylalkyl optionally substituted with 1 or 2 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, C_2-C_4 alkanoyl, phenyl C_1-C_4 alkyl, and $-SO_2$ C_1-C_4 alkyl;

alkenyl; alkynyl; heteroaryl optionally substituted with 1, 2, or 3 groups that are independently OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, NH_2, $NH(C_1$-C_6 alkyl) or $N(C_1$-C_6 alkyl)(C_1-C_6 alkyl); heteroarylalkyl optionally substituted with 1, 2, or 3 groups that are independently C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, NH_2, $NH(C_1$-C_6 alkyl) or $N(C_1$-C_6 alkyl)(C_1-C_6 alkyl); phenyl; C_3-C_8 cycloalkyl, and cycloalkylalkyl, wherein the phenyl; C_3-C_8 cycloalkyl, and cycloalkylalkyl groups are optionally substituted with 1, 2, 3, 4 or 5 groups that are independently halogen, CN, NO_2, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_6 alkanoyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, hydroxy, C_1-C_6 hydroxyalkyl, C_1-C_6 alkoxy C_1-C_6 alkyl, C_1-C_6 thioalkoxy, C_1-C_6 thioalkoxy C_1-C_6 alkyl, or C_1-C_6 alkoxy C_1-C_6 alkoxy; and

Y is C_1-C_{10} alkyl optionally substituted with 1 thru 3 substituents which can be the same or different and are selected from halogen, hydroxy, alkoxy, thioalkoxy, and haloalkoxy.

Preferred compounds of formula I, formula I-1 and formula I-2 include compounds of formula I-3, i.e., compounds of formula I, I-1 or I-2 wherein:

R_1 is $(CH_2)_{n1}$-$(R_{1\text{-aryl}})$ where n_1 is zero or one and $R_{1\text{-aryl}}$ is phenyl optionally substituted with 1, 2, 3, or 4 groups independently selected from C_1-C_6 alkyl optionally substituted with 1, 2, or 3 substituents selected from the group consisting of C_1-C_3 alkyl, halogen, -OH, -SH, $-NR_{1\text{-a}}R_{1\text{-b}}$, -C≡N, $-CF_3$, and C_1-C_3 alkoxy; halogen; C_1-C_6 alkoxy; $-NR_{N\text{-}2}R_{N\text{-}3}$; and OH; wherein

$R_{1\text{-a}}$ and $R_{1\text{-b}}$ are -H or C_1-C_6 alkyl;

$R_{N\text{-}2}$ and $R_{N\text{-}3}$ at each occurrence are independently selected from the group consisting of $-C_1$-C_8 alkyl optionally substituted with 1, 2, or 3 groups independently

selected from the group consisting of -OH, $-NH_2$, phenyl and halogen; $-C_3-C_8$ cycloalkyl; $-(C_1-C_2$ alkyl)-$(C_3-C_8$ cycloalkyl); $-(C_1-C_6$ alkyl)-O-$(C_1-C_3$ alkyl); $-C_2-C_6$ alkenyl; $-C_2-C_6$ alkynyl; $-C_1-C_6$ alkyl chain with one double bond and one triple bond; aryl; heteroaryl; heterocycloalkyl; or

R_{N-2}, R_{N-3} and the nitrogen to which they are attached form a 5, 6, or 7 membered heterocycloalkyl or heteroaryl group, wherein said heterocycloalkyl or heteroaryl group is optionally fused to a benzene, pyridine, or pyrimidine ring, and said groups are unsubstituted or substituted with 1, 2, 3, 4, or 5 groups that at each occurrence are independently C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, halo C_1-C_6 alkyl, halo C_1-C_6 alkoxy, -CN, $-NO_2$, $-NH_2$, $NH(C_1-C_6$ alkyl), $N(C_1-C_6$ alkyl)$(C_1-C_6$ alkyl), -OH, $-C(O)NH_2$, $-C(O)NH(C_1-C_6$ alkyl), $-C(O)N(C_1-C_6$ alkyl)$(C_1-C_6$ alkyl), C_1-C_6 alkoxy C_1-C_6 alkyl, C_1-C_6 thioalkoxy, and C_1-C_6 thioalkoxy C_1-C_6 alkyl.

Preferred compounds of formula I-3 include compounds wherein:

R_1 is aryl, heteroaryl, heterocyclyl, $-C_1-C_6$ alkyl-aryl, $-C_1-C_6$ alkyl-heteroaryl, or $-C_1-C_6$ alkyl-heterocyclyl, where the ring portions of each are optionally substituted with 1, 2, 3, or 4 groups independently selected from halogen, -OH, -SH, $-C\equiv N$, $-NO_2$, $-NR_{105}R'_{105}$, $-CO_2R$, -N(R)COR', or $-N(R)SO_2R'$ (where R_{105}, R'_{105}, R and R' are as defined above), $-C(=O)-(C_1-C_4)$ alkyl, $-SO_2$-amino, $-SO_2$-mono or dialkylamino, -C(=O)-amino, -C(=O)-mono or dialkylamino, $-SO_2-(C_1-C_4)$ alkyl, or

C_1-C_6 alkoxy optionally substituted with 1, 2, or 3 groups which are independently selected from halogen, or

C_3-C_7 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, -CF_3, C_1-C_3 alkoxy, amino, -C_1-C_6 alkyl and mono- or dialkylamino, or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, -CF_3, -C_1-C_3 alkoxy, amino, mono- or dialkylamino and -C_1-C_3 alkyl, or

C_2-C_{10} alkenyl or C_2-C_{10} alkynyl each of which is optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, -CF_3, C_1-C_3 alkoxy, amino, C_1-C_6 alkyl and mono- or dialkylamino; and the heterocyclyl group is optionally further substituted with oxo.

Preferred compounds of formula I-3 include compounds wherein:

R_1 is -C_1-C_6 alkyl-aryl, -C_1-C_6 alkyl-heteroaryl, or -C_1-C_6 alkyl-heterocyclyl, where the ring portions of each are optionally substituted with 1, 2, 3, or 4 groups independently selected from halogen, -OH, -SH, -C≡N, -NO_2, -$NR_{105}R'_{105}$, -CO_2R, -N(R)COR', or -N(R)SO_2R' (where R_{105}, R'_{105}, R and R' are as defined above), -C(=O)-(C_1-C_4) alkyl, -SO_2-amino, -SO_2-mono or dialkylamino, -C(=O)-amino, -C(=O)-mono or dialkylamino, -SO_2-(C_1-C_4) alkyl, or

C_1-C_6 alkoxy optionally substituted with 1, 2, or 3 groups which are independently selected from halogen, or

C_3-C_7 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen,

-OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, amino, $-C_1$-C_6 alkyl and mono- or dialkylamino, or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, $-CF_3$, $-C_1$-C_3 alkoxy, amino, mono- or dialkylamino and $-C_1$-C_3 alkyl, or

C_2-C_{10} alkenyl or C_2-C_{10} alkynyl each of which is optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, amino, C_1-C_6 alkyl and mono- or dialkylamino; and the heterocyclyl group is optionally further substituted with oxo.

Preferred compounds of formula I-3 include compounds wherein:

R_1 is $-(CH_2)$-aryl, $-(CH_2)$-heteroaryl, or $-(CH_2)$-heterocyclyl, where the ring portions of each are optionally substituted with 1, 2, 3, or 4 groups independently selected from halogen, -OH, -SH, -C≡N, $-NO_2$, $-NR_{105}R'_{105}$, $-CO_2R$, -N(R)COR', or $-N(R)SO_2R'$ (where R_{105}, R'_{105}, R and R' are as defined above), $-C(=O)-(C_1$-$C_4)$ alkyl, $-SO_2$-amino, $-SO_2$-mono or dialkylamino, -C(=O)-amino, -C(=O)-mono or dialkylamino, $-SO_2-(C_1$-$C_4)$ alkyl, or

C_1-C_6 alkoxy optionally substituted with 1, 2, or 3 groups which are independently selected from halogen, or

C_3-C_7 cycloalkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, -C≡N, $-CF_3$, C_1-C_3 alkoxy, amino, $-C_1$-C_6 alkyl and mono- or dialkylamino, or

C_1-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from halogen, -

OH, -SH, $-C\equiv N$, $-CF_3$, $-C_1-C_3$ alkoxy, amino, mono- or dialkylamino and $-C_1-C_3$ alkyl, or

C_2-C_{10} alkenyl or C_2-C_{10} alkynyl each of which is optionally substituted with 1, 2, or 3 groups independently selected from halogen, -OH, -SH, $-C\equiv N$, $-CF_3$, C_1-C_3 alkoxy, amino, C_1-C_6 alkyl and mono- or dialkylamino; and the heterocyclyl group is optionally further substituted with oxo.

Preferred compounds of formula I-3 include compounds wherein:

R_1 is $-CH_2$-phenyl or $-CH_2$-pyridinyl where the ring portions of each are optionally substituted with 1, 2, 3, or 4 groups independently selected from halogen, C_1-C_4 alkoxy, hydroxy, $-NO_2$, and

C_1-C_4 alkyl optionally substituted with 1, 2, or 3 substituents independently selected from halogen, OH, SH, NH_2, $NH(C_1-C_6$ alkyl), $N-(C_1-C_6$ alkyl)(C_1-C_6 alkyl), $C\equiv N$, CF_3.

Preferred compounds of formula I-3 include compounds wherein: R_1 is $-CH_2$-phenyl or $-CH_2$-pyridinyl where the phenyl or pyridinyl rings are each optionally substituted with 1 or 2 groups independently selected from halogen, C_1-C_2 alkyl, C_1-C_2 alkoxy, hydroxy, $-CF_3$, and $-NO_2$.

Preferred compounds of formula I-3 include compounds wherein: R_1 is $-CH_2$-phenyl where the phenyl ring is optionally substituted with 2 groups independently selected from halogen, C_1-C_2 alkyl, C_1-C_2 alkoxy, hydroxy, and $-NO_2$.

Preferred compounds of formula I-3 include compounds wherein: R_1 is benzyl, or 3,5-difluorobenzyl.

Preferred compounds of formula I-1, I-2, and I-3 also include compounds of formula I-4, i.e., those of formula I-1, I-2, or I-3 wherein:

R_C is hydrogen, $-(CR_{245}R_{250})_{0-4}$-aryl, $-(CR_{245}R_{250})_{0-4}$-heteroaryl, $-(CR_{245}R_{250})_{0-4}$-heterocyclyl,

C_2-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from the group consisting of R_{205}, R_{110}, R_{120}, R_{130}, $-OC{=}ONR_{235}R_{240}$, $-S(=O)_{0-2}(C_1$-C_6 alkyl), -SH, and $-S(=O)_2NR_{235}R_{240}$,

$-(CH_2)_{0-3}-(C_3$-$C_8)$ cycloalkyl wherein the cycloalkyl is optionally substituted with 1, 2, or 3 groups independently selected from the group consisting of R_{205}, $-CO_2H$, and $-CO_2-(C_1$-C_4 alkyl), or

C_2-C_{10} alkenyl or C_2-C_{10} alkynyl, each of which is optionally substituted with 1, 2, or 3 independently selected R_{205} groups, wherein

each aryl and heteroaryl is optionally substituted with 1, 2, or 3 R_{200}, and wherein each heterocyclyl is optionally substituted with 1, 2, 3, or 4 independently selected R_{210} groups.

Preferred compounds of formula I-4 also include compounds wherein

R_C is $-(CR_{245}R_{250})$-aryl, $-(CR_{245}R_{250})$-heteroaryl, $-(CR_{245}R_{250})$-heterocyclyl,

C_2-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from the group consisting of R_{205}, R_{110}, R_{120}, R_{130}, $-OC{=}ONR_{235}R_{240}$, $-S(=O)_{0-2}(C_1$-C_6 alkyl), -SH, and $-S(=O)_2NR_{235}R_{240}$, wherein

each aryl and heteroaryl is optionally substituted with 1, 2, or 3 R_{200}, and wherein each heterocyclyl is optionally substituted with 1, 2, 3, or 4 independently selected R_{210} groups.

Preferred compounds of formula I-4 also include compounds wherein

R_C is $-(CH_2)$-aryl, $-(CH_2)$-heteroaryl, or

C_2-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from C_1-C_6 alkyl, halogen,

-OH, -O-phenyl, -SH, -S-C_1-C_6 alkyl, -C≡N, -CF_3, C_1-C_6 alkoxy, and NH_2, wherein

each aryl and heteroaryl is optionally substituted with 1, 2, or 3 groups selected from OH, -NO_2, halogen, -CO_2H, C≡N, -$(CH_2)_{0-4}$-CO-$NR_{220}R_{225}$, -$(CH_2)_{0-4}$-CO-(C_1-C_{12} alkyl), and -$(CH_2)_{0-4}$-SO_2-$NR_{220}R_{225}$.

Preferred compounds of formula I-4 also include compounds wherein

R_C is -(CH_2)-phenyl, wherein phenyl is optionally substituted with 1, 2, or 3 groups selected from OH, -NO_2, halogen, -CO_2H, and C≡N, or

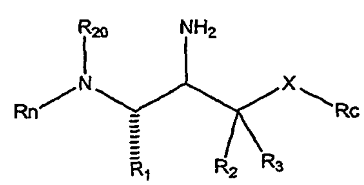
R_C is C_2-C_{10} alkyl optionally substituted with 1, 2, or 3 groups independently selected from C_1-C_6 alkyl, halogen, -OH, -C≡N, -CF_3, C_1-C_6 alkoxy, and NH_2.

Preferred compounds of formula I-4 also include compounds wherein R_C is benzyl, ethyl, n-propyl, iso-propyl, n-butyl, t-butyl or iso-butyl.

In a preferred embodiment, X is O. In another preferred embodiment, X is S. In yet another preferred embodiment, X is NR_{20}, where R_{20} is as defined above. In an alternative preferred embodiment, X is $NR_{20}NR_{20}$, where each R_{20} is independently as defined above.

In another preferred embodiment, each of R_2, R_3 and R_{20} are independently hydrogen.

A preferred stereochemistry for compounds of formula I is as follows:

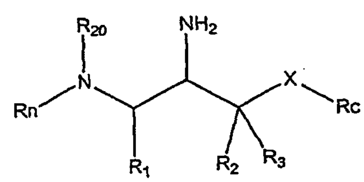


The invention encompasses a method of treating a patient who has, or in preventing a patient from getting, a disease or condition selected from the group consisting of Alzheimer's disease, for helping prevent or delay the onset of Alzheimer's

disease, for treating patients with mild cognitive impairment (MCI) and preventing or delaying the onset of Alzheimer's disease in those who would progress from MCI to AD, for treating Down's syndrome, for treating humans who have Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-Type, for treating cerebral amyloid angiopathy and preventing its potential consequences, i.e. single and recurrent lobar hemorrhages, for treating other degenerative dementias, including dementias of mixed vascular and degenerative origin, dementia associated with Parkinson's disease, dementia associated with progressive supranuclear palsy, dementia associated with cortical basal degeneration, diffuse Lewy body type of Alzheimer's disease and who is in need of such treatment which comprises administration of a therapeutically effective amount of a compound selected from the group consisting of a substituted aminoether of the formula (I), or a pharmaceutically acceptable salt or ester thereof.

Preferably, the patient is a human. More preferably, the disease is Alzheimer's disease. More preferably, the disease is dementia.

The invention includes methods for making compounds of formula (I)



(I)

or a pharmaceutically acceptable salt or ester thereof, wherein X, R_{20}, R_1, R_2, R_3, Rn and Rc are as defined above or below.

The invention is the compounds of formula (I), which are useful in treating and preventing Alzheimer's disease. The anti-Alzheimer's compounds of formula (I) are made by methods

well known to those skilled in the art from starting compounds known to those skilled in the art. The process chemistry is well known to those skilled in the art. The most general process to prepare compounds of formula (I) of the invention is set forth in Scheme 1. One skilled in the art will appreciate that these are all wellknown reactions in organic chemistry. A chemist skilled in the art, knowing the chemical structure of the biologically end product of formula (I) of the invention would be able to prepare them by known methods from known starting materials without any additional information. The explanation below therefore is not necessary but is deemed helpful to those skilled in the art who desire to make the compounds of the invention.

Scheme 1 sets forth a general method used in the invention to prepare compounds of formula (I). Preparation of the starting epoxide of formula (II), i.e., the Boc-3,5-difluorophenylalanine epoxide, was adapted from the procedure of Luly, JR, et al. *J. Org. Chem.* **1987**, *52*, 1487-1492. The starting material utilized in the preparation of Boc-3,5-difluorophenylalanine *threo* epoxide was Boc protected l-3,5-difluorophenylalanine available from Synthetech, Inc. (1290 Industrial Way, P. O. Box 646, Albany, OR 97321 USA). *Tetrahedron Lett.*, 38, 3175 (1997) further discloses a process for the preparation of N-BOC protected epoxides from protected amino acid esters.

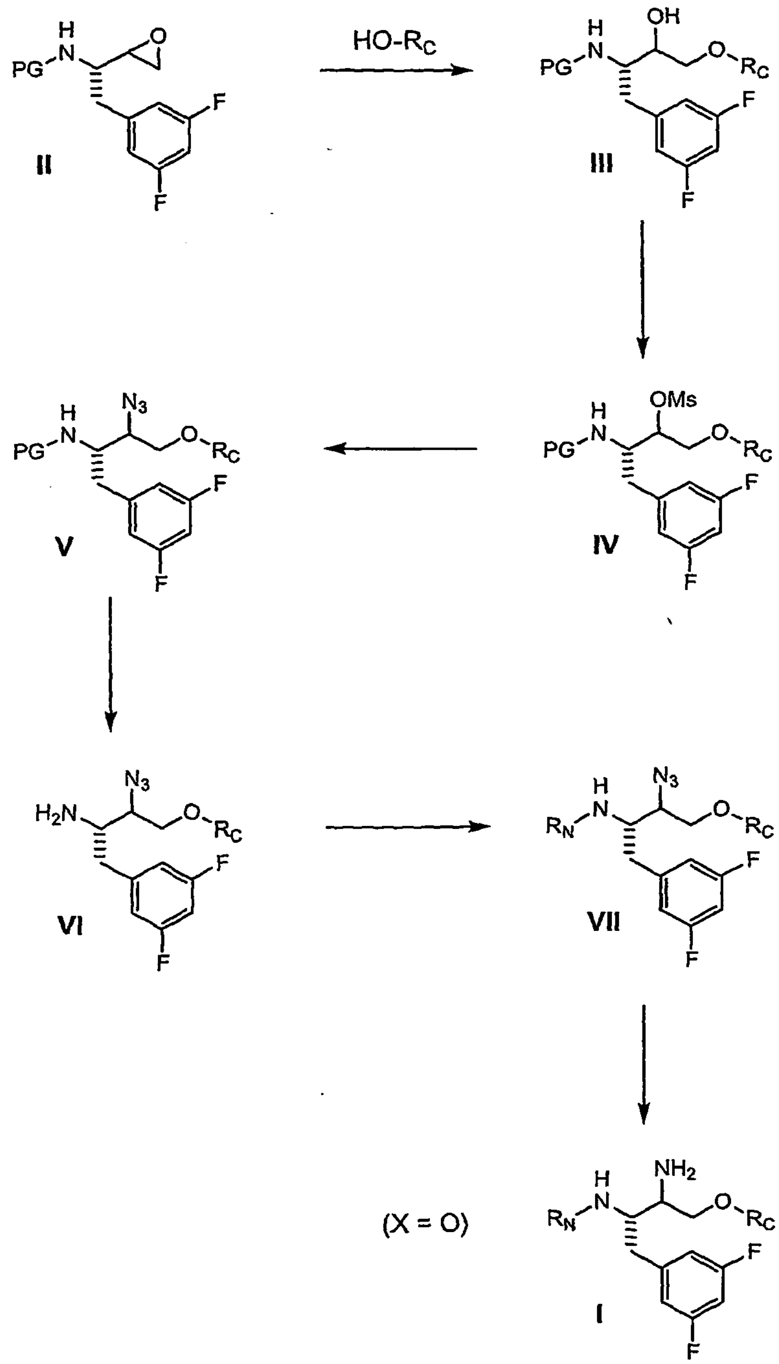
As used herein, PG means "protecting group", and is typically the BOC protecting group (tBu-O-C(=O)-) or the CBZ protecting group (Ph-CH_2-O-C(=O)-) may be treated with alcohols of formula (HO-R_C) to afford ring opened products of formula (III). Reactions may be run in organic solvents such as tetrahydrofuran, dimethylsulfoxide, or dimethylformamide at temperatures ranging from room temperature up to the reflux point of the solvent employed. The resulting compound of formula (III) may be isolated by conventional means such as

crystallization or chromatography, or it may be used directly in the next step of a synthesis of this invention.

Compounds of formula (III) may be converted to compounds of formula (IV) in the presence of methanesulfonyl chloride in an organic solvent, optimally dichloromethane, in the presence of a base such as di-isopropylethylamine or more preferably triethylamine at a temperature ranging from -78°C (dry ice/acetone) to room temperature.

Compounds of formula (IV) may be converted to compounds of formula (V) by treatment with reagents such as sodium azide in an organic solvent such as dimethylsulfoxide or, more preferably, dimethylformamide at a temperature range of about 50°C to about 120°C. One skilled in synthetic organic chemistry will understand the optimal combination of conditions necessary to effect the desired reaction.

Scheme I: Preparation of substituted aminoethers



Compounds of formula (V) may be converted to compounds of formula (VI) via deprotection by means known to those

skilled in the art for removal of amine protecting group. Suitable means for removal of the amine-protecting group depends on the nature of the protecting group. Those skilled in the art, knowing the nature of a specific protecting group, know which reagent is preferable for its removal. For example, it is preferred to remove the preferred protecting group, BOC, by dissolving compound of formula (V) in a trifluoroacetic acid/dichloromethane (1/1) mixture. When complete, the solvents are removed under reduced pressure to give the corresponding amine (as the corresponding salt, i.e. trifluoroacetic acid salt) which is used without further purification. However, if desired, the amine can be purified further by means well known to those skilled in the art, such as for example, recrystallization. Further, if the non-salt form is desired that also can be obtained by means known to those skilled in the art, such as for example, preparing the free base amine via treatment of the salt with mild basic conditions. Additional BOC deprotection conditions and deprotection conditions for other protecting groups can be found in T.W. Green and P.G.M. Wuts in "Protective Groups in Organic Chemistry, John Wiley and Sons, 1991, p. 309. There are numerous methods to remove the representative benzyl-protecting group on the phenol. Preferred methods can be found in T.W. Green and P.G.M. Wuts in "Protecting Groups in Organic Chemistry, John Wiley and Sons, 1999, p. 86.

Compounds of formula (VI) may be converted to compounds of formula (VII) with an appropriately substituted amide forming agent to produce coupled amides by nitrogen-acylation means known to those skilled in the art. Nitrogen acylation conditions for reaction of the amine (VI) with an amide forming agent to produce the corresponding substituted amide (VII) are known to those skilled in the art and can be found in R.C. Larock in Comprehensive Organic Transformations, VCH Publishers, 1989, p. 981, 979, and 972. The nitrogen-

acylation of primary amines to produce secondary amides is one of the oldest known reactions. The amide forming agents are readily prepared according to known starting materials by methods known in the literature. Amide forming regents are represented by R_N-X_2 wherein X_2 comprises -OH (carboxylic acid) or halide (acyl halide), preferably chlorine, or a suitable group to produce a mixed anhydride.

Compounds of formula (VII) may be converted to compounds of formula (I), wherein X=O, in an organic solvent such as methanol or more preferably ethanol in the presence of a catalyst such as 10% palladium on carbon. The reaction is carried our under 1 atmosphere of hydrogen to effect the reduction.

Where it is desired to prepare compounds of formula (I) wherein X is -S-, -NH-, or -NH-NR_A-, one may simply replace the nucleophile HO-R_C in the conversion of compound of formula (II) to compound of formula (III) with the appropriate and desired sulfur or amine nucleophile and following the remaining methods set forth in Scheme 1.

When the amino protecting group is no longer needed, it is removed by methods well known to those skilled in the art. By definition the amino protecting group must be readily removable as is known to those skilled in the art by methods well known to those skilled in the art. Suitable amino protecting group is selected from the group consisting of t-butoxycarbonyl, benzyloxycarbonyl, formyl, trityl, acetyl, trichloroacetyl, dichloroacetyl, chloroacetyl, trifluoroacetyl, difluoroacetyl, fluoroacetyl, 4-phenylbenzyloxycarbonyl, 2-methylbenzyloxycarbonyl, 4-ethoxybenzyloxycarbonyl, 4-fluorobenzyloxycarbonyl, 4-chlorobenzyloxycarbonyl, 3-chlorobenzyloxycarbonyl, 2-chlorobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, 4-bromobenzyloxycarbonyl, 3-bromobenzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, 4-cyanobenzyloxycarbonyl, 2-(4-

xenyl)isopropoxycarbonyl, 1,1-diphenyleth-1-yloxycarbonyl, 1,1-diphenylprop-1-yloxycarbonyl, 2-phenylprop-2-yloxycarbonyl, 2-(p-toluyl)prop-2-yloxycarbonyl, cyclopentanyloxycarbonyl, 1-methylcyclopentanyloxycarbonyl, cyclohexanyloxycarbonyl, 1-methylcyclohexanyloxycabonyl, 2-methylcyclohexanyloxycarbonyl, 2-(4-toluylsulfonyl)ethoxycarbonyl, 2-(methylsulfonyl)ethoxycarbonyl, 2-(triphenylphosphino)ethoxycarbonyl, fluorenylmethoxycarbonyl, 2-(trimethylsilyl)ethoxycarbonyl, allyloxycarbonyl, 1-(trimethylsilylmethyl)prop-1-enyloxycarbonyl, 5-benzisoxalylmethoxycarbonyl, 4-acetoxybenzyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, 2-ethynyl-2-propoxycarbonyl, cyclopropylmethoxycarbonyl, 4-(decyloxyl)benzyloxycarbonyl, isobornyloxycarbonyl and 1-piperidyloxycarbonyl, 9-fluorenylmethyl carbonate,
$-CH-CH=CH_2$ and phenyl-C(=N-)-H.

Suitable means for removal of the amine-protecting group depends on the nature of the protecting group. Those skilled in the art, knowing the nature of a specific protecting group, know which reagent is preferable for its removal. For example, it is preferred to remove the preferred protecting group, BOC, by dissolving the protected material in a trifluoroacetic acid/dichloromethane mixture.

The compounds of the invention may contain geometric or optical isomers as well as tautomers. Thus, the invention includes all tautomers and pure geometric isomers, such as the *E* and *Z* geometric isomers, as well as mixtures thereof. Futhermore, the invention includes pure enantiomers and diasteriomers as well as mixtures thereof, including racemic mixtures. The individual geometric isomers, enantiomers, or diasteriomers may be prepared or isolated by methods known in the art.

Pharmaceutically acceptable salts are any salt which retains the activity of the parent compound and does not impart any deleterious or undesirable effect on the subject to whom it is administered and in the context in which it is administered. Pharmaceutically acceptable salts include salts of both inorganic and organic acids. The preferred pharmaceutically acceptable salts include salts of the following acids acetic, aspartic, benzenesulfonic, benzoic, bicarbonic, bisulfuric, bitartaric, butyric, calcium edetate, camsylic, carbonic, chlorobenzoic, citric, edetic, edisylic, estolic, esyl, esylic, formic, fumaric, gluceptic, gluconic, glutamic, glycollylarsanilic, hexamic, hexylresorcinoic, hydrabamic, hydrobromic, hydrochloric, hydroiodic, hydroxynaphthoic, isethionic, lactic, lactobionic, maleic, malic, malonic, mandelic, methanesulfonic, methylnitric, methylsulfuric, mucic, muconic, napsylic, nitric, oxalic, p-nitromethanesulfonic, pamoic, pantothenic, phosphoric, monohydrogen phosphoric, dihydrogen phosphoric, phthalic, polygalactouronic, propionic, salicylic, stearic, succinic, succinic, sulfamic, sulfanilic, sulfonic, sulfuric, tannic, tartaric, teoclic and toluenesulfonic. For other acceptable salts, see *Int. J. Pharm.*, 33, 201-217 (1986) and *J.Pharm.Sci.*, 66(1), 1, (1977).

The invention provides compounds, compositions, kits, and methods for inhibiting beta-secretase enzyme activity and A beta peptide production. Inhibition of beta-secretase enzyme activity halts or reduces the production of A beta from APP and reduces or eliminates the formation of beta-amyloid deposits in the brain.

Methods of the Invention

The compounds of the invention, and pharmaceutically acceptable salts thereof, are useful for treating humans or animals suffering from a condition characterized by a pathological form of beta-amyloid peptide, such as beta-

amyloid plaques, and for helping to prevent or delay the onset of such a condition. For example, the compounds are useful for treating Alzheimer's disease, for helping prevent or delay the onset of Alzheimer's disease, for treating patients with MCI (mild cognitive impairment) and preventing or delaying the onset of Alzheimer's disease in those who would progress from MCI to AD, for treating Down's syndrome, for treating humans who have Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-Type, for treating cerebral amyloid angiopathy and preventing its potential consequences, i.e. single and recurrent lobal hemorrhages, for treating other degenerative dementias, including dementias of mixed vascular and degenerative origin, dementia associated with Parkinson's disease, dementia associated with progressive supranuclear palsy, dementia associated with cortical basal degeneration, and diffuse Lewy body type Alzheimer's disease. The compounds and compositions of the invention are particularly useful for treating or preventing Alzheimer's disease. When treating or preventing these diseases, the compounds of the invention can either be used individually or in combination, as is best for the patient.

As used herein, the term "treating" means that the compounds of the invention can be used in humans with at least a tentative diagnosis of disease. The compounds of the invention will delay or slow the progression of the disease thereby giving the individual a more useful life span.

The term "preventing" means that the compounds of the invention are useful when administered to a patient who has not been diagnosed as possibly having the disease at the time of administration, but who would normally be expected to develop the disease or be at increased risk for the disease. The compounds of the invention will slow the development of disease symptoms, delay the onset of the disease, or prevent the individual from developing the disease at all. Preventing

also includes administration of the compounds of the invention to those individuals thought to be predisposed to the disease due to age, familial history, genetic or chromosomal abnormalities, and/or due to the presence of one or more biological markers for the disease, such as a known genetic mutation of APP or APP cleavage products in brain tissues or fluids.

In treating or preventing the above diseases, the compounds of the invention are administered in a therapeutically effective amount. The therapeutically effective amount will vary depending on the particular compound used and the route of administration, as is known to those skilled in the art.

In treating a patient displaying any of the diagnosed above conditions a physician may administer a compound of the invention immediately and continue administration indefinitely, as needed. In treating patients who are not diagnosed as having Alzheimer's disease, but who are believed to be at substantial risk for Alzheimer's disease, the physician should preferably start treatment when the patient first experiences early pre-Alzheimer's symptoms such as, memory or cognitive problems associated with aging. In addition, there are some patients who may be determined to be at risk for developing Alzheimer's through the detection of a genetic marker such as APOE4 or other biological indicators that are predictive for Alzheimer's disease. In these situations, even though the patient does not have symptoms of the disease, administration of the compounds of the invention may be started before symptoms appear, and treatment may be continued indefinitely to prevent or delay the outset of the disease.

Dosage Forms and Amounts

The compounds of the invention can be administered orally, parenternally, (IV, IM, depo-IM, SQ, and depo SQ),

sublingually, intranasally (inhalation), intrathecally, topically, or rectally. Dosage forms known to those of skill in the art are suitable for delivery of the compounds of the invention.

Compositions are provided that contain therapeutically effective amounts of the compounds of the invention. The compounds are preferably formulated into suitable pharmaceutical preparations such as tablets, capsules, or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. Typically the compounds described above are formulated into pharmaceutical compositions using techniques and procedures well known in the art.

About 1 to 500 mg of a compound or mixture of compounds of the invention or a physiologically acceptable salt or ester is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in those compositions or preparations is such that a suitable dosage in the range indicated is obtained. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 2 to about 100 mg, more preferably about 10 to about 30 mg of the active ingredient. The term "unit dosage from" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

To prepare compositions, one or more compounds of the invention are mixed with a suitable pharmaceutically acceptable carrier. Upon mixing or addition of the compound(s), the resulting mixture may be a solution, suspension, emulsion, or the like. Liposomal suspensions may

also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for lessening or ameliorating at least one symptom of the disease, disorder, or condition treated and may be empirically determined.

Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration. In addition, the active materials can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, or have another action. The compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients.

Where the compounds exhibit insufficient solubility, methods for solubilizing may be used. Such methods are known and include, but are not limited to, using cosolvents such as dimethylsulfoxide (DMSO), using surfactants such as Tween®, and dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as salts or prodrugs may also be used in formulating effective pharmaceutical compositions.

The concentration of the compound is effective for delivery of an amount upon administration that lessens or ameliorates at least one symptom of the disorder for which the compound is administered. Typically, the compositions are formulated for single dosage administration.

The compounds of the invention may be prepared with carriers that protect them against rapid elimination from the body, such as time-release formulations or coatings. Such

carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems. The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in known *in vitro* and *in vivo* model systems for the treated disorder.

The compounds and compositions of the invention can be enclosed in multiple or single dose containers. The enclosed compounds and compositions can be provided in kits, for example, including component parts that can be assembled for use. For example, a compound inhibitor in lyophilized form and a suitable diluent may be provided as separated components for combination prior to use. A kit may include a compound inhibitor and a second therapeutic agent for co-administration. The inhibitor and second therapeutic agent may be provided as separate component parts. A kit may include a plurality of containers, each container holding one or more unit dose of the compound of the invention. The containers are preferably adapted for the desired mode of administration, including, but not limited to tablets, gel capsules, sustained-release capsules, and the like for oral administration; depot products, pre-filled syringes, ampules, vials, and the like for parenternal administration; and patches, medipads, creams, and the like for topical administration.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the active compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

If oral administration is desired, the compound should be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

Oral compositions will generally include an inert diluent or an edible carrier and may be compressed into tablets or enclosed in gelatin capsules. For the purpose of oral therapeutic administration, the active compound or compounds can be incorporated with excipients and used in the form of tablets, capsules, or troches. Pharmaceutically compatible binding agents and adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches, and the like can contain any of the following ingredients or compounds of a similar nature: a binder such as, but not limited to, gum

tragacanth, acacia, corn starch, or gelatin; an excipient such as microcrystalline cellulose, starch, or lactose; a disintegrating agent such as, but not limited to, alginic acid and corn starch; a lubricant such as, but not limited to, magnesium stearate; a gildant, such as, but not limited to, colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; and a flavoring agent such as peppermint, methyl salicylate, or fruit flavoring.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials, which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings, and flavors.

The active materials can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action.

Solutions or suspensions used for parenternal, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent such as water for injection, saline solution, fixed oil, a naturally occurring vegetable oil such as sesame oil, coconut oil, peanut oil, cottonseed oil, and the like, or a synthetic fatty vehicle such as ethyl oleate, and the like, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antimicrobial agents such as benzyl alcohol and methyl parabens; antioxidants such as ascorbic acid and sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates, and phosphates; and agents for the adjustment of tonicity such as

sodium chloride and dextrose. Parenternal preparations can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass, plastic, or other suitable material. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

Where administered intravenously, suitable carriers include physiological saline, phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents such as glucose, polyethylene glycol, polypropyleneglycol, and mixtures thereof. Liposomal suspensions including tissue-targeted liposomes may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known for example, as described in U.S. Patent No. 4,522,811.

The active compounds may be prepared with carriers that protect the compound against rapid elimination from the body, such as time-release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers such as collagen, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid, and the like. Methods for preparation of such formulations are known to those skilled in the art.

The compounds of the invention can be administered orally, parenternally (IV, IM, depo-IM, SQ, and depo-SQ), sublingually, intranasally (inhalation), intrathecally, topically, or rectally. Dosage forms known to those skilled in the art are suitable for delivery of the compounds of the invention.

Compounds of the invention may be administered enterally or parenterally. When administered orally, compounds of the invention can be administered in usual dosage forms for oral administration as is well known to those skilled in the art.

These dosage forms include the usual solid unit dosage forms of tablets and capsules as well as liquid dosage forms such as solutions, suspensions, and elixirs. When the solid dosage forms are used, it is preferred that they be of the sustained release type so that the compounds of the invention need to be administered only once or twice daily.

The oral dosage forms are administered to the patient 1, 2, 3, or 4 times daily. It is preferred that the compounds of the invention be administered either three or fewer times, more preferably once or twice daily. Hence, it is preferred that the compounds of the invention be administered in oral dosage form. It is preferred that whatever oral dosage form is used, that it be designed so as to protect the compounds of the invention from the acidic environment of the stomach. Enteric coated tablets are well known to those skilled in the art. In addition, capsules filled with small spheres each coated to protect from the acidic stomach, are also well known to those skilled in the art.

When administered orally, an administered amount therapeutically effective to inhibit beta-secretase activity, to inhibit A beta production, to inhibit A beta deposition, or to treat or prevent AD is from about 0.1 mg/day to about 1,000 mg/day. It is preferred that the oral dosage is from about 1 mg/day to about 100 mg/day. It is more preferred that the oral dosage is from about 5 mg/day to about 50 mg/day. It is understood that while a patient may be started at one dose, that dose may be varied over time as the patient's condition changes.

Compounds of the invention may also be advantageously delivered in a nano crystal dispersion formulation. Preparation of such formulations is described, for example, in U.S. Patent 5,145,684. Nano crystalline dispersions of HIV protease inhibitors and their method of use are described in

US 6,045,829. The nano crystalline formulations typically afford greater bioavailability of drug compounds.

The compounds of the invention can be administered parenterally, for example, by IV, IM, depo-IM, SC, or depo-SC. When administered parenterally, a therapeutically effective amount of about 0.5 to about 100 mg/day, preferably from about 5 to about 50 mg daily should be delivered. When a depot formulation is used for injection once a month or once every two weeks, the dose should be about 0.5 mg/day to about 50 mg/day, or a monthly dose of from about 15 mg to about 1,500 mg. In part because of the forgetfulness of the patients with Alzheimer's disease, it is preferred that the parenteral dosage form be a depo formulation.

The compounds of the invention can be administered sublingually. When given sublingually, the compounds of the invention should be given one to four times daily in the amounts described above for IM administration.

The compounds of the invention can be administered intranasally. When given by this route, the appropriate dosage forms are a nasal spray or dry powder, as is known to those skilled in the art. The dosage of the compounds of the invention for intranasal administration is the amount described above for IM administration.

The compounds of the invention can be administered intrathecally. When given by this route the appropriate dosage form can be a parenternal dosage form as is known to those skilled in the art. The dosage of the compounds of the invention for intrathecal administration is the amount described above for IM administration.

The compounds of the invention can be administered topically. When given by this route, the appropriate dosage form is a cream, ointment, or patch. Because of the amount of the compounds of the invention to be administered, the patch is preferred. When administered topically, the dosage is from

about 0.5 mg/day to about 200 mg/day. Because the amount that can be delivered by a patch is limited, two or more patches may be used. The number and size of the patch is not important, what is important is that a therapeutically effective amount of the compounds of the invention be delivered as is known to those skilled in the art. The compounds of the invention can be administered rectally by suppository as is known to those skilled in the art. When administered by suppository, the therapeutically effective amount is from about 0.5 mg to about 500 mg.

The compounds of the invention can be administered by implants as is known to those skilled in the art. When administering a compound of the invention by implant, the therapeutically effective amount is the amount described above for depot administration.

The invention here is the new compounds of the invention and new methods of using the compounds of the invention. Given a particular compound of the invention and a desired dosage form, one skilled in the art would know how to prepare and administer the appropriate dosage form.

The compounds of the invention are used in the same manner, by the same routes of administration, using the same pharmaceutical dosage forms, and at the same dosing schedule as described above, for preventing disease or treating patients with MCI (mild cognitive impairment) and preventing or delaying the onset of Alzheimer's disease in those who would progress from MCI to AD, for treating or preventing Down's syndrome, for treating humans who have Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-Type, for treating cerebral amyloid angiopathy and preventing its potential consequences, i.e. single and recurrent lobar hemorrhages, for treating other degenerative dementias, including dementias of mixed vascular and degenerative origin, dementia associated with Parkinson's disease, dementia

associated with progressive supranuclear palsy, dementia associated with cortical basal degeneration, and diffuse Lewy body type of Alzheimer's disease.

The compounds of the invention can be used in combination, with each other or with other therapeutic agents or approaches used to treat or prevent the conditions listed above. Such agents or approaches include: acetylcholine esterase inhibitors such as tacrine (tetrahydroaminoacridine, marketed as COGNEX®), donepezil hydrochloride, (marketed as Aricept® and rivastigmine (marketed as Exelon®); gamma-secretase inhibitors; anti-inflammatory agents such as cyclooxygenase II inhibitors; anti-oxidants such as Vitamin E and ginkolides; immunological approaches, such as, for example, immunization with A beta peptide or administration of anti-A beta peptide antibodies; statins; and direct or indirect neurotropic agents such as Cerebrolysin®, AIT-082 (Emilieu, 2000, *Arch. Neurol.* 57:454), and other neurotropic agents of the future.

It should be apparent to one skilled in the art that the exact dosage and frequency of administration will depend on the particular compounds of the invention administered, the particular condition being treated, the severity of the condition being treated, the age, weight, general physical condition of the particular patient, and other medication the individual may be taking as is well known to administering physicians who are skilled in this art.

Inhibition of APP Cleavage

The compounds of the invention inhibit cleavage of APP between Met595 and Asp596 numbered for the APP695 isoform, or a mutant thereof, or at a corresponding site of a different isoform, such as APP751 or APP770, or a mutant thereof (sometimes referred to as the "beta secretase site"). While not wishing to be bound by a particular theory, inhibition of beta-secretase activity is thought to inhibit production of

beta amyloid peptide (A beta). Inhibitory activity is demonstrated in one of a variety of inhibition assays, whereby cleavage of an APP substrate in the presence of a beta-secretase enzyme is analyzed in the presence of the inhibitory compound, under conditions normally sufficient to result in cleavage at the beta-secretase cleavage site. Reduction of APP cleavage at the beta-secretase cleavage site compared with an untreated or inactive control is correlated with inhibitory activity. Assay systems that can be used to demonstrate efficacy of the compound inhibitors of the invention are known. Representative assay systems are described, for example, in U.S. Patents No. 5,942,400, 5,744,346, as well as in the Examples below.

The enzymatic activity of beta-secretase and the production of A beta can be analyzed *in vitro* or *in vivo*, using natural, mutated, and/or synthetic APP substrates, natural, mutated, and/or synthetic enzyme, and the test compound. The analysis may involve primary or secondary cells expressing native, mutant, and/or synthetic APP and enzyme, animal models expressing native APP and enzyme, or may utilize transgenic animal models expressing the substrate and enzyme. Detection of enzymatic activity can be by analysis of one or more of the cleavage products, for example, by immunoassay, flurometric or chromogenic assay, HPLC, or other means of detection. Inhibitory compounds are determined as those having the ability to decrease the amount of beta-secretase cleavage product produced in comparison to a control, where beta-secretase mediated cleavage in the reaction system is observed and measured in the absence of inhibitory compounds.

Beta-secretase

Various forms of beta-secretase enzyme are known, and are available and useful for assay of enzyme activity and inhibition of enzyme activity. These include native, recombinant, and synthetic forms of the enzyme. Human beta-

secretase is known as Beta Site APP Cleaving Enzyme (BACE), Asp2, and memapsin 2, and has been characterized, for example, in U.S. Patent No. 5,744,346 and published PCT patent applications WO98/22597, WO00/03819, WO01/23533, and WO00/17369, as well as in literature publications (Hussain et.al., 1999, *Mol.Cell.Neurosci.* 14:419-427; Vassar et.al., 1999, *Science* 286:735-741; Yan et.al., 1999, *Nature* 402:533-537; Sinha et.al., 1999, *Nature* 40:537-540; and Lin et.al., 2000, *PNAS USA* 97:1456-1460). Synthetic forms of the enzyme have also been described (WO98/22597 and WO00/17369). Beta-secretase can be extracted and purified from human brain tissue and can be produced in cells, for example mammalian cells expressing recombinant enzyme.

Preferred compounds are effective to inhibit 50% of beta-secretase enzymatic activity at a concentration of less than about 50 micromolar, preferably at a concentration of less than about 10 micromolar, more preferably less than about 1 micromolar, and most preferably less than about 10 nanomolar.

APP Substrate

Assays that demonstrate inhibition of beta-secretase-mediated cleavage of APP can utilize any of the known forms of APP, including the 695 amino acid "normal" isotype described by Kang et.al., 1987, *Nature* 325:733-6, the 770 amino acid isotype described by Kitaguchi et. al., 1981, *Nature* 331:530-532, and variants such as the Swedish Mutation (KM670-1NL) (APP-SW), the London Mutation (V7176F), and others. See, for example, U.S. Patent No. 5,766,846 and also Hardy, 1992, *Nature Genet.* 1:233-234, for a review of known variant mutations. Additional useful substrates include the dibasic amino acid modification, APP-KK disclosed, for example, in WO 00/17369, fragments of APP, and synthetic peptides containing the beta-secretase cleavage site, wild type (WT) or mutated

form, e.g., SW, as described, for example, in U.S. Patent No 5,942,400 and WO00/03819.

The APP substrate contains the beta-secretase cleavage site of APP (KM-DA or NL-DA) for example, a complete APP peptide or variant, an APP fragment, a recombinant or synthetic APP, or a fusion peptide. Preferably, the fusion peptide includes the beta-secretase cleavage site fused to a peptide having a moiety useful for enzymatic assay, for example, having isolation and/or detection properties. A useful moiety may be an antigenic epitope for antibody binding, a label or other detection moiety, a binding substrate, and the like.

Antibodies

Products characteristic of APP cleavage can be measured by immunoassay using various antibodies, as described, for example, in Pirttila et.al., 1999, *Neuro.Lett.* 249:21-4, and in U.S. Pat. No. 5,612,486. Useful antibodies to detect A beta include, for example, the monoclonal antibody 6E10 (Senetek, St. Louis, MO) that specifically recognizes an epitope on amino acids 1-16 of the A beta peptide; antibodies 162 and 164 (New York State Institute for Basic Research, Staten Island, NY) that are specific for human A beta 1-40 and 1-42, respectively; and antibodies that recognize the junction region of beta-amyloid peptide, the site between residues 16 and 17, as described in U.S. Patent No. 5,593,846. Antibodies raised against a synthetic peptide of residues 591 to 596 of APP and SW192 antibody raised against 590-596 of the Swedish mutation are also useful in immunoassay of APP and its cleavage products, as described in U.S. Patent Nos. 5,604,102 and 5,721,130.

Assay Systems

Assays for determining APP cleavage at the beta-secretase cleavage site are well known in the art. Exemplary assays,

are described, for example, in U.S. Patent Nos. 5,744,346 and 5,942,400, and described in the Examples below.

Cell Free Assays

Exemplary assays that can be used to demonstrate the inhibitory activity of the compounds of the invention are described, for example, in WO00/17369, WO 00/03819, and U.S. Patents No. 5,942,400 and 5,744,346. Such assays can be performed in cell-free incubations or in cellular incubations using cells expressing a beta-secretase and an APP substrate having a beta-secretase cleavage site.

An APP substrate containing the beat-secretase cleavage site of APP, for example, a complete APP or variant, an APP fragment, or a recombinant or synthetic APP substrate containing the amino acid sequence: KM-DA or NL-DA, is incubated in the presence of beta-secretase enzyme, a fragment thereof, or a synthetic or recombinant polypeptide variant having beta-secretase activity and effective to cleave the beta-secretase cleavage site of APP, under incubation conditions suitable for the cleavage activity of the enzyme. Suitable substrates optionally include derivatives that may be fusion proteins or peptides that contain the substrate peptide and a modification useful to facilitate the purification or detection of the peptide or its beta-secretase cleavage products. Useful modifications include the insertion of a known antigenic epitope for antibody binding; the linking of a label or detectable moiety, the linking of a binding substrate, and the like.

Suitable incubation conditions for a cell-free *in vitro* assay include, for example: approximately 200 nanomolar to 10 micromolar substrate, approximately 10 to 200 picomolar enzyme, and approximately 0.1 nanomolar to 10 micromolar inhibitor compound, in aqueous solution, at an approximate pH of 4 -7, at approximately 37 degrees C, for a time period of

approximately 10 minutes to 3 hours. These incubation conditions are exemplary only, and can be varied as required for the particular assay components and/or desired measurement system. Optimization of the incubation conditions for the particular assay components should account for the specific beta-secretase enzyme used and its pH optimum, any additional enzymes and/or markers that might be used in the assay, and the like. Such optimization is routine and will not require undue experimentation.

One useful assay utilizes a fusion peptide having maltose binding protein (MBP) fused to the C-terminal 125 amino acids of APP-SW. The MBP portion is captured on an assay substrate by anti-MBP capture antibody. Incubation of the captured fusion protein in the presence of beta-secretase results in cleavage of the substrate at the beta-secretase cleavage site. Analysis of the cleavage activity can be, for example, by immunoassay of cleavage products. One such immunoassay detects a unique epitope exposed at the carboxy terminus of the cleaved fusion protein, for example, using the antibody SW192. This assay is described, for example, in U.S. Patent No 5,942,400.

Cellular Assay

Numerous cell-based assays can be used to analyze beta-secretase activity and/or processing of APP to release A beta. Contact of an APP substrate with a beta-secretase enzyme within the cell and in the presence or absence of a compound inhibitor of the invention can be used to demonstrate beta-secretase inhibitory activity of the compound. Preferably, assay in the presence of a useful inhibitory compound provides at least about 30%, most preferably at least about 50% inhibition of the enzymatic activity, as compared with a non-inhibited control.

In one embodiment, cells that naturally express beta-secretase are used. Alternatively, cells are modified to

express a recombinant beta-secretase or synthetic variant enzyme as discussed above. The APP substrate may be added to the culture medium and is preferably expressed in the cells. Cells that naturally express APP, variant or mutant forms of APP, or cells transformed to express an isoform of APP, mutant or variant APP, recombinant or synthetic APP, APP fragment, or synthetic APP peptide or fusion protein containing the beta-secretase APP cleavage site can be used, provided that the expressed APP is permitted to contact the enzyme and enzymatic cleavage activity can be analyzed.

Human cell lines that normally process A beta from APP provide a useful means to assay inhibitory activities of the compounds of the invention. Production and release of A beta and/or other cleavage products into the culture medium can be measured, for example by immunoassay, such as Western blot or enzyme-linked immunoassay (EIA) such as by ELISA.

Cells expressing an APP substrate and an active beta-secretase can be incubated in the presence of a compound inhibitor to demonstrate inhibition of enzymatic activity as compared with a control. Activity of beta-secretase can be measured by analysis of one or more cleavage products of the APP substrate. For example, inhibition of beta-secretase activity against the substrate APP would be expected to decrease release of specific beta-secretase induced APP cleavage products such as A beta.

Although both neural and non-neural cells process and release A beta, levels of endogenous beta-secretase activity are low and often difficult to detect by EIA. The use of cell types known to have enhanced beta-secretase activity, enhanced processing of APP to A beta, and/or enhanced production of A beta are therefore preferred. For example, transfection of cells with the Swedish Mutant form of APP (APP-SW); with APP-KK; or with APP-SW-KK provides cells having enhanced beta-

secretase activity and producing amounts of A beta that can be readily measured.

In such assays, for example, the cells expressing APP and beta-secretase are incubated in a culture medium under conditions suitable for beta-secretase enzymatic activity at its cleavage site on the APP substrate. On exposure of the cells to the compound inhibitor, the amount of A beta released into the medium and/or the amount of CTF99 fragments of APP in the cell lysates is reduced as compared with the control. The cleavage products of APP can be analyzed, for example, by immune reactions with specific antibodies, as discussed above.

Preferred cells for analysis of beta-secretase activity include primary human neuronal cells, primary transgenic animal neuronal cells where the transgene is APP, and other cells such as those of a stable 293 cell line expressing APP, for example, APP-SW.

In *Vivo* Assays: Animal Models

Various animal models can be used to analyze beta-secretase activity and /or processing of APP to release A beta, as described above. For example, transgenic animals expressing APP substrate and beta-secretase enzyme can be used to demonstrate inhibitory activity of the compounds of the invention. Certain transgenic animal models have been described, for example, in U.S. Patent Nos: 5,877,399; 5,612,486; 5,387,742; 5,720,936; 5,850,003; 5,877,015,, and 5,811,633, and in Ganes et.al., 1995, *Nature* 373:523. Preferred are animals that exhibit characteristics associated with the pathophysiology of AD. Administration of the compound inhibitors of the invention to the transgenic mice described herein provides an alternative method for demonstrating the inhibitory activity of the compounds. Administration of the compounds in a pharmaceutically effective carrier and via an administrative route that reaches

the target tissue in an appropriate therapeutic amount is also preferred.

Inhibition of beta-secretase mediated cleavage of APP at the beta-secretase cleavage site and of A beta release can be analyzed in these animals by measure of cleavage fragments in the animal's body fluids such as cerebral fluid or tissues. Analysis of brain tissues for A beta deposits or plaques is preferred.

On contacting an APP substrate with a beta-secretase enzyme in the presence of an inhibitory compound of the invention and under conditions sufficient to permit enzymatic mediated cleavage of APP and/or release of A beta from the substrate, the compounds of the invention are effective to reduce beta-secretase-mediated cleavage of APP at the beta-secretase cleavage site and/or effective to reduce released amounts of A beta. Where such contacting is the administration of the inhibitory compounds of the invention to an animal model, for example, as described above, the compounds are effective to reduce A beta deposition in brain tissues of the animal, and to reduce the number and/or size of beta amyloid plaques. Where such administration is to a human subject, the compounds are effective to inhibit or slow the progression of disease characterized by enhanced amounts of A beta, to slow the progression of AD in the, and/or to prevent onset or development of AD in a patient at risk for the disease.

Unless defined otherwise, all scientific and technical terms used herein have the same meaning as commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are hereby incorporated by reference for all purposes.

Definitions and Conventions

The definitions and explanations below are for the terms as used throughout this entire document including both the specification and the claims.

Definitions

All temperatures are in degrees Celsius.

TLC refers to thin-layer chromatography.

psi refers to pounds/in^2.

HPLC refers to high pressure liquid chromatography.

THF refers to tetrahydrofuran.

DMF refers to dimethylformamide.

EDC refers to ethyl-1-(3-dimethylaminopropyl)carbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

HOBt refers to 1-hydroxy benzotriazole hydrate.

NMM refers to N-methylmorpholine.

NBS refers to N-bromosuccinimide.

TEA refers to triethylamine.

BOC refers to 1,1-dimethylethoxy carbonyl or t-butoxycarbonyl,

represneted schematically as-CO-O-C(CH_3)$_3$.

CBZ refers to benzyloxycarbonyl, -CO-O-CH_2-ϕ.

FMOC refers to 9-fluorenylmethyl carbonate.

TFA refers to trifluoracetic acid, CF_3-COOH.

CDI refers to 1,1'-carbonyldiimidazole.

Saline refers to an aqueous saturated sodium chloride solution.

Chromatography (column and flash chromatography) refers to purification/separation of compounds expressed as (support, eluent). It is understood that the appropriate fractions are pooled and concentrated to give the desired compound(s).

CMR refers to C-13 magnetic resonance spectroscopy, chemical shifts are reported in ppm (δ) downfield from TMS.

NMR refers to nuclear (proton) magnetic resonance spectroscopy, chemical shifts are reported in ppm (d) downfield from TMS.

IR refers to infrared spectroscopy.

-phenyl refers to phenyl (C_6H_5).

MS refers to mass spectrometry expressed as m/e, m/z or mass/charge unit. MH^+ refers to the positive ion of a parent plus a hydrogen atom. EI refers to electron impact. CI refers to chemical ionization. FAB refers to fast atom bombardment.

HRMS refers to high resolution mass spectrometry.

Ether refers to diethyl ether.

When solvent pairs are used, the ratios of solvents used are volume/volume (v/v).

When the solubility of a solid in a solvent is used the ratio of the solid to the solvent is weight/volume (wt/v).

BOP refers to benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate.

TBDMSCl refers to *t*-butyldimethylsilyl chloride.

TBDMSOTf refers to *t*-butyldimethylsilyl trifluosulfonic acid ester.

Trisomy 21 refers to Down's Syndrome.

APP, amyloid precursor protein, is defined as any APP polypeptide, including APP variants, mutations, and isoforms, for example, as disclosed in U.S. Patent No. 5,766,846.

A beta, amyloid beta peptide, is defined as any peptide resulting from beta-secretase mediated cleavage of APP, including peptides of 39, 40, 41, 42, and 43 amino acids, and extending from the beta-secretase cleavage site to amino acids 39, 40, 41, 42, or 43.

Beta-secretase (BACE1, Asp2, Memapsin 2) is an aspartyl protease that mediates cleavage of APP at the amino-terminal edge of A beta. Human beta-secretase is described, for example, in WO00/17369.

By "alkyl" and "C_1-C_6 alkyl" in the invention is meant straight or branched chain alkyl groups having 1-6 carbon atoms, such as, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, isopentyl, neopentyl, hexyl, 2-hexyl, 3-hexyl, and 3-methylpentyl. It is understood that in cases where an alkyl chain of a substituent (e.g. of an alkyl, alkoxy or alkenyl group) is shorter or longer than 6 carbons, it will be so indicated in the second "C" as, for example, "C_1-C_{10}" indicates a maximum of 10 carbons.

By "alkoxy" and "C_1-C_6 alkoxy" in the invention is meant straight or branched chain alkyl groups having 1-6 carbon atoms, attached through at least one divalent oxygen atom, such as, for example, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, pentoxy, isopentoxy, neopentoxy, hexoxy, and 3-methylpentoxy.

By the term "halogen" in the invention is meant fluorine, bromine, chlorine, and iodine.

"Alkenyl" and "C_2-C_6 alkenyl" means straight and branched hydrocarbon radicals having from 2 to 6 carbon atoms and from one to three double bonds and includes, for example, ethenyl, propenyl, 1-but-3-enyl, 1-pent-3-enyl, 1-hex-5-enyl and the like.

"Alkynyl" and "C_2-C_6 alkynyl" means straight and branched hydrocarbon radicals having from 2 to 6 carbon atoms and one or two triple bonds and includes ethynyl, propynyl, butynyl, pentyn-2-yl and the like.

As used herein, the term "cycloalkyl" refers to saturated carbocyclic radicals having three to twelve carbon atoms. The cycloalkyl can be monocyclic, or a polycyclic fused system. Examples of such radicals include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl. The cycloalkyl groups herein are unsubstituted or, as specified, substituted in one or more substitutable positions with various groups. For example, such cycloalkyl groups may be optionally

substituted with C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, hydroxy, cyano, nitro, amino, mono(C_1-C_6)alkylamino, di(C_1-C_6)alkylamino, C_2-C_6alkenyl, C_2-C_6alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, amino(C_1-C_6)alkyl, mono(C_1-C_6)alkylamino(C_1-C_6)alkyl or di(C_1-C_6)alkylamino(C_1-C_6)alkyl.

By "aryl" is meant an aromatic carbocyclic group having a single ring (e.g., phenyl), multiple rings (e.g., biphenyl), or multiple condensed rings in which at least one is aromatic, (e.g., 1,2,3,4-tetrahydronaphthyl, naphthyl), which is optionally mono-, di-, or trisubstituted. Preferred aryl groups of the invention are phenyl, 1-naphthyl, 2-naphthyl, indanyl, indenyl, dihydronaphthyl, tetralinyl or 6,7,8,9-tetrahydro-5H-benzo[a]cycloheptenyl. The aryl groups herein are unsubstituted or, as specified, substituted in one or more substitutable positions with various groups. For example, such aryl groups may be optionally substituted with, for example, C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, hydroxy, cyano, nitro, amino, mono(C_1-C_6)alkylamino, di(C_1-C_6)alkylamino, C_2-C_6alkenyl, C_2-C_6alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, amino(C_1-C_6)alkyl, mono(C_1-C_6)alkylamino(C_1-C_6)alkyl, di(C_1-C_6)alkylamino(C_1-C_6)alkyl, -COOH, -C(=O)O(C_1-C_6 alkyl), -C(=O)NH_2, -C(=O)N(mono- or di-C_1-C_6 alkyl), -S(C_1-C_6 alkyl), -SO_2(C_1-C_6 alkyl), -O-C(=O)(C_1-C_6 alkyl), -NH-C(=O)-(C_1-C_6 alkyl), -N(C_1-C_6 alkyl)-C(=O)-(C_1-C_6 alkyl), -NH-SO_2-(C_1-C_6 alkyl), -N(C_1-C_6 alkyl)-SO_2-(C_1-C_6 alkyl), -NH-C(=O)NH_2, -NH-C(=O)N(mono- or di-C_1-C_6 alkyl), -NH(C_1-C_6 alkyl)-C(=O)-NH_2 or -NH(C_1-C_6 alkyl)-C(=O)-N-(mono- or di-C_1-C_6 alkyl).

By "heteroaryl" is meant one or more aromatic ring systems of 5-, 6-, or 7-membered rings which includes fused ring systems of 9-11 atoms containing at least one and up to four heteroatoms selected from nitrogen, oxygen, or sulfur. Preferred heteroaryl groups of the invention include pyridinyl, pyrimidinyl, quinolinyl, benzothienyl, indolyl, indolinyl, pryidazinyl, pyrazinyl, isoindolyl, isoquinolyl,

quinazolinyl, quinoxalinyl, phthalazinyl, imidazolyl, isoxazolyl, pyrazolyl, oxazolyl, thiazolyl, indolizinyl, indazolyl, benzothiazolyl, benzimidazolyl, benzofuranyl, furanyl, thienyl, pyrrolyl, oxadiazolyl, thiadiazolyl, triazolyl, tetrazolyl, oxazolopyridinyl, imidazopyridinyl, isothiazolyl, naphthyridinyl, cinnolinyl, carbazolyl, beta-carbolinyl, isochromanyl, chromanyl, tetrahydroisoquinolinyl, isoindolinyl, isobenzotetrahydrofuranyl, isobenzotetrahydrothienyl, isobenzothienyl, benzoxazolyl, pyridopyridinyl, benzotetrahydrofuranyl, benzotetrahydrothienyl, purinyl, benzodioxolyl, triazinyl, phenoxazinyl, phenothiazinyl, pteridinyl, benzothiazolyl, imidazopyridinyl, imidazothiazolyl, dihydrobenzisoxazinyl, benzisoxazinyl, benzoxazinyl, dihydrobenzisothiazinyl, benzopyranyl, benzothiopyranyl, coumarinyl, isocoumarinyl, chromonyl, chromanonyl, pyridinyl-N-oxide, tetrahydroquinolinyl, dihydroquinolinyl, dihydroquinolinonyl, dihydroisoquinolinonyl, dihydrocoumarinyl, dihydroisocoumarinyl, isoindolinonyl, benzodioxanyl, benzoxazolinonyl, pyrrolyl N-oxide,, pyrimidinyl N-oxide, pyridazinyl N-oxide, pyrazinyl N-oxide, quinolinyl N-oxide, indolyl N-oxide, indolinyl N-oxide, isoquinolyl N-oxide, quinazolinyl N-oxide, quinoxalinyl N-oxide, phthalazinyl N-oxide, imidazolyl N-oxide, isoxazolyl N-oxide, oxazolyl N-oxide, thiazolyl N-oxide, indolizinyl N-oxide, indazolyl N-oxide, benzothiazolyl N-oxide, benzimidazolyl N-oxide, pyrrolyl N-oxide, oxadiazolyl N-oxide, thiadiazolyl N-oxide, triazolyl N-oxide, tetrazolyl N-oxide,. benzothiopyranyl S-oxide, benzothiopyranyl S,S-dioxide. The heteroaryl groups herein are unsubstituted or, as specified, substituted in one or more substitutable positions with various groups. For example, such heteroaryl groups may be optionally substituted with C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, hydroxy, cyano, nitro, amino, mono(C_1-C_6)alkylamino, di(C_1-C_6)alkylamino, C_2-C_6alkenyl, C_2-

C_6alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, amino(C_1-C_6)alkyl, mono(C_1-C_6)alkylamino(C_1-C_6)alkyl or di(C_1-C_6)alkylamino(C_1-C_6)alkyl, -COOH, -C(=O)O(C_1-C_6 alkyl), -C(=O)NH_2, -C(=O)N(mono- or di-C_1-C_6 alkyl), -S(C_1-C_6 alkyl), -SO_2(C_1-C_6 alkyl), -O-C(=O)(C_1-C_6 alkyl), -NH-C(=O)-(C_1-C_6 alkyl), -N(C_1-C_6 alkyl)-C(=O)-(C_1-C_6 alkyl), -NH-SO_2-(C_1-C_6 alkyl), -N(C_1-C_6 alkyl)-SO_2-(C_1-C_6 alkyl), -NH-C(=O)NH_2, -NH-C(=O)N(mono- or di-C_1-C_6 alkyl), -NH(C_1-C_6 alkyl)-C(=O)-NH_2 or -NH(C_1-C_6 alkyl)-C(=O)-N-(mono- or di-C_1-C_6 alkyl).

By "heterocycle", "heterocycloalkyl" or "heterocyclyl" is meant one or more carbocyclic ring systems of 4-, 5-, 6-, or 7-membered rings which includes fused ring systems of 9-11 atoms containing at least one and up to four heteroatoms selected from nitrogen, oxygen, or sulfur. Preferred heterocycles of the invention include morpholinyl, thiomorpholinyl, thiomorpholinyl S-oxide, thiomorpholinyl S,S-dioxide, piperazinyl, homopiperazinyl, pyrrolidinyl, pyrrolinyl, tetrahydropyranyl, piperidinyl, tetrahydrofuranyl, tetrahydrothienyl, homopiperidinyl, homomorpholinyl, homothiomorpholinyl, homothiomorpholinyl S,S-dioxide, oxazolidinonyl, dihydropyrazolyl, dihydropyrrolyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrimidinyl, dihydrofuryl, dihydropyranyl, tetrahydrothienyl S-oxide, tetrahydrothienyl S,S-dioxide and homothiomorpholinyl S-oxide. The heterocycle groups herein are unsubstituted or, as specified, substituted in one or more substitutable positions with various groups. For example, such heterocycle groups may be optionally substituted with C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, hydroxy, cyano, nitro, amino, mono(C_1-C_6)alkylamino, di(C_1-C_6)alkylamino, C_2-C_6alkenyl, C_2-C_6alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, amino(C_1-C_6)alkyl, mono(C_1-C_6)alkylamino(C_1-C_6)alkyl, di(C_1-C_6)alkylamino(C_1-C_6)alkyl or =O.

"Pharmaceutically acceptable" refers to those properties and/or substances that are acceptable to the patient from a

pharmacological/toxicological point of view and to the manufacturing pharmaceutical chemist from a physical/chemical point of view regarding composition, formulation, stability, patient acceptance and bioavailability.

A therapeutically effective amount is defined as an amount effective to reduce or lessen at least one symptom of the disease being treated or to reduce or delay onset of one or more clinical markers or symptoms of the disease.

It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition containing "a compound" includes a mixture of two or more compounds. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

Unless defined otherwise, all scientific and technical terms used herein have the same meaning as commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are hereby incorporated by reference for all purposes.

BIOLOGY EXAMPLES

Example A

Enzyme Inhibition Assay

The compounds of the invention are analyzed for inhibitory activity by use of the MBP-C125 assay. This assay determines the relative inhibition of beta-secretase cleavage of a model APP substrate, MBP-C125SW, by the compounds assayed as compared with an untreated control. A detailed description of the assay parameters can be found, for example, in U.S. Patent No. 5,942,400. Briefly, the substrate is a fusion peptide formed of maltose binding protein (MBP) and the carboxy terminal 125 amino acids of APP-SW, the Swedish

mutation. The beta-secretase enzyme is derived from human brain tissue as described in Sinha et.al, 1999, *Nature* 40:537-540) or recombinantly produced as the full-length enzyme (amino acids 1-501), and can be prepared, for example, from 293 cells expressing the recombinant cDNA, as described in WO00/47618. Human brain □-Secretase from concentrated HiQ pool prepared 7/16/97 in 0.20% Triton was used in the assay. Inhibition of the enzyme is analyzed, for example, by immunoassay of the enzyme's cleavage products. One exemplary ELISA uses an anti-MBP capture antibody that is deposited on precoated and blocked 96-well high binding plates, followed by incubation with diluted enzyme reaction supernatant, incubation with a specific reporter antibody, for example, biotinylated anti-SW192 reporter antibody, and further incubation with streptavidin/alkaline phosphatase. In the assay, cleavage of the intact MBP-C125SW fusion protein results in the generation of a truncated amino-terminal fragment, exposing a new SW-192 antibody-positive epitope at the carboxy terminus. Detection is effected by a fluorescent substrate signal on cleavage by the phosphatase. ELISA only detects cleavage following Leu 596 at the substrate's APP-SW 751 mutation site.

Specific Assay Procedure:

Compounds are diluted in a 1:1 dilution series to a six-point concentration curve (two wells per concentration) in one 96-plate row per compound tested. Each of the test compounds is prepared in DMSO to make up a 10 millimolar stock solution. The stock solution is serially diluted in DMSO to obtain a final compound concentration of 200 micromolar at the high point of a 6-point dilution curve. Ten (10) microliters of each dilution is added to each of two wells on row C of a corresponding V-bottom plate to which 190 microliters of 52 millimolar NaOAc, 7.9% DMSO, pH 4.5 are pre-added. The NaOAc diluted compound plate is spun down to pellet precipitant and

20 microliters/well is transferred to a corresponding flat-bottom plate to which 30 microliters of ice-cold enzyme-substrate mixture (2.5 microliters MBP-C125SW substrate, 0.03 microliters enzyme and 24.5 microliters ice cold 0.09% TX100 per 30 microliters) is added. The final reaction mixture of 200 micromolar compound at the highest curve point is in 5% DMSO, 20 millimolar NaAc, 0.06% TX100, at pH 4.5.

Warming the plates to 37 degrees C starts the enzyme reaction. After 90 minutes at 37 degrees C, 200 microliters/well cold specimen diluent is added to stop the reaction and 20 microliters/well was transferred to a corresponding anti-MBP antibody coated ELISA plate for capture, containing 80 microliters/well specimen diluent. This reaction is incubated overnight at 4 degrees C and the ELISA is developed the next day after a 2 hour incubation with anti-192SW antibody, followed by Streptavidin-AP conjugate and fluorescent substrate. The signal is read on a fluorescent plate reader.

Relative compound inhibition potency is determined by calculating the concentration of compound that showed a fifty percent reduction in detected signal (IC_{50}) compared to the enzyme reaction signal in the control wells with no added compound.

Example B

Cell Free Inhibition Assay Utilizing a Synthetic APP Substrate

A synthetic APP substrate that can be cleaved by beta-secretase and having N-terminal biotin and made fluorescent by the covalent attachment of Oregon green at the Cys residue is used to assay beta-secretase activity in the presence or absence of the inhibitory compounds of the invention. Useful substrates include the following:

Biotin-SEVNL-DAEFRC[oregon green]KK [SEQ ID NO: 1]

Biotin-SEVKM-DAEFRC[oregon green]KK [SEQ ID NO: 2]

Biotin-GLNIKTEEISEISY-EVEFRC[oregon green]KK [SEQ ID NO: 3]

Biotin-ADRGLTTRPGSGLTNIKTEEISEVNL-DAEFRC[oregon green]KK [SEQ ID NO:4]

Biotin-FVNQHLCoxGSHLVEALY-LVCoxGERGFFYTPKAC[oregon green]KK [SEQ ID NO: 5]

The enzyme (0.1 nanomolar) and test compounds (0.001 - 100 micromolar) are incubated in pre-blocked, low affinity, black plates (384 well) at 37 degrees for 30 minutes. The reaction is initiated by addition of 150 millimolar substrate to a final volume of 30 microliter per well. The final assay conditions are: 0.001 - 100 micromolar compound inhibitor; 0.1 molar sodium acetate (pH 4.5); 150 nanomolar substrate; 0.1 nanomolar soluble beta-secretase; 0.001% Tween 20, and 2% DMSO. The assay mixture is incubated for 3 hours at 37 degrees C, and the reaction is terminated by the addition of a saturating concentration of immunopure streptavidin. After incubation with streptavidin at room temperature for 15 minutes, fluorescence polarization is measured, for example, using a LJL Acqurest (Ex485 nm/ Em530 nm). The activity of the beta-secretase enzyme is detected by changes in the fluorescence polarization that occur when the substrate is cleaved by the enzyme. Incubation in the presence or absence of compound inhibitor demonstrates specific inhibition of beta-secretase enzymatic cleavage of its synthetic APP substrate. In this assay, preferred compounds of the invention exhibit an IC_{50} of less than 50 micromolar.

Example C

Beta-Secretase Inhibition: P26-P4'SW Assay

Synthetic substrates containing the beta-secretase cleavage site of APP are used to assay beta-secretase activity, using the methods described, for example, in published PCT application WO00/47618. The P26-P4'SW substrate is a peptide of the sequence:

(biotin)CGGADRGLTTRPGSGLTNIKTEEISEVNLDAEF [SEQ ID NO: 6]

The P26-P1 standard has the sequence:

(biotin)CGGADRGLTTRPGSGLTNIKTEEISEVNL [SEQ ID NO: 7].

Briefly, the biotin-coupled synthetic substrates are incubated at a concentration of from about 0 to about 200 micromolar in this assay. When testing inhibitory compounds, a substrate concentration of about 1.0 micromolar is preferred. Test compounds diluted in DMSO are added to the reaction mixture, with a final DMSO concentration of 5%. Controls also contain a final DMSO concentration of 5%. The concentration of beta secretase enzyme in the reaction is varied, to give product concentrations with the linear range of the ELISA assay, about 125 to 2000 picomolar, after dilution.

The reaction mixture also includes 20 millimolar sodium acetate, pH 4.5, 0.06% Triton X100, and is incubated at 37 degrees C for about 1 to 3 hours. Samples are then diluted in assay buffer (for example, 145.4 nanomolar sodium chloride, 9.51 millimolar sodium phosphate, 7.7 millimolar sodium azide, 0.05% Triton X405, 6g/liter bovine serum albumin, pH 7.4) to quench the reaction, then diluted further for immunoassay of the cleavage products.

Cleavage products can be assayed by ELISA. Diluted samples and standards are incubated in assay plates coated with capture antibody, for example, SW192, for about 24 hours at 4 degrees C. After washing in TTBS buffer (150 millimolar sodium chloride, 25 millimolar Tris, 0.05% Tween 20, pH 7.5), the samples are incubated with streptavidin-AP according to the manufacturer's instructions. After a one hour incubation

at room temperature, the samples are washed in TTBS and incubated with fluorescent substrate solution A (31.2 g/liter 2-amino-2-methyl-1-propanol, 30 mg/liter, pH 9.5). Reaction with streptavidin-alkaline phosphate permits detection by fluorescence. Compounds that are effective inhibitors of beta-secretase activity demonstrate reduced cleavage of the substrate as compared to a control.

Example D

Assays using Synthetic Oligopeptide-Substrates

Synthetic oligopeptides are prepared that incorporate the known cleavage site of beta-secretase, and optionally detectable tags, such as fluorescent or chromogenic moieties. Examples of such peptides, as well as their production and detection methods are described in U.S. Patent No: 5,942,400, herein incorporated by reference. Cleavage products can be detected using high performance liquid chromatography, or fluorescent or chromogenic detection methods appropriate to the peptide to be detected, according to methods well known in the art.

By way of example, one such peptide has the sequence SEVNL-DAEF [SEQ ID NO: 8], and the cleavage site is between residues 5 and 6. Another preferred substrate has the sequence ADRGLTTRPGSGLTNIKTEEISEVNL-DAEF [SEQ ID NO: 9], and the cleavage site is between residues 26 and 27.

These synthetic APP substrates are incubated in the presence of beta-secretase under conditions sufficient to result in beta-secretase mediated cleavage of the substrate. Comparison of the cleavage results in the presence of the compound inhibitor to control results provides a measure of the compound's inhibitory activity.

Example E

Inhibition of Beta-Secretase Activity - Cellular Assay

An exemplary assay for the analysis of inhibition of beta-secretase activity utilizes the human embryonic kidney cell line HEKp293 (ATCC Accession No. CRL-1573) transfected with APP751 containing the naturally occurring double mutation Lys651Met52 to Asn651Leu652 (numbered for APP751), commonly called the Swedish mutation and shown to overproduce A beta (Citron et al., 1992, *Nature* 360:672-674), as described in U.S. Patent No. 5,604,102.

The cells are incubated in the presence/absence of the inhibitory compound (diluted in DMSO) at the desired concentration, generally up to 10 micrograms/ml. At the end of the treatment period, conditioned media is analyzed for beta-secretase activity, for example, by analysis of cleavage fragments. A beta can be analyzed by immunoassay, using specific detection antibodies. The enzymatic activity is measured in the presence and absence of the compound inhibitors to demonstrate specific inhibition of beta-secretase mediated cleavage of APP substrate.

Example F

Inhibition of Beta-Secretase in Animal Models of AD

Various animal models can be used to screen for inhibition of beta-secretase activity. Examples of animal models useful in the invention include, but are not limited to, mouse, guinea pig, dog, and the like. The animals used can be wild type, transgenic, or knockout models. In addition, mammalian models can express mutations in APP, such as APP695-SW and the like described herein. Examples of transgenic non-human mammalian models are described in U.S. Patent Nos. 5,604,102, 5,912,410 and 5,811,633.

PDAPP mice, prepared as described in Games et al., 1995, *Nature* 373:523-527 are useful to analyze *in vivo* suppression of A beta release in the presence of putative inhibitory compounds. As described in U.S. Patent No. 6,191,166, 4 month

old PDAPP mice are administered compound formulated in vehicle, such as corn oil. The mice are dosed with compound (1-30 mg/ml; preferably 1-10 mg/ml). After time, e.g., 3-10 hours, the animals are sacrificed, and brains removed for analysis.

Transgenic animals are administered an amount of the compound inhibitor formulated in a carrier suitable for the chosen mode of administration. Control animals are untreated, treated with vehicle, or treated with an inactive compound. Administration can be acute, i.e., single dose or multiple doses in one day, or can be chronic, i.e., dosing is repeated daily for a period of days. Beginning at time 0, brain tissue or cerebral fluid is obtained from selected animals and analyzed for the presence of APP cleavage peptides, including A beta, for example, by immunoassay using specific antibodies for A beta detection. At the end of the test period, animals are sacrificed and brain tissue or cerebral fluid is analyzed for the presence of A beta and/or beta-amyloid plaques. The tissue is also analyzed for necrosis.

Animals administered the compound inhibitors of the invention are expected to demonstrate reduced A beta in brain tissues or cerebral fluids and reduced beta amyloid plaques in brain tissue, as compared with non-treated controls.

Example G

Inhibition of A Beta Production in Human Patients

Patients suffering from Alzheimer's Disease (AD) demonstrate an increased amount of A beta in the brain. AD patients are administered an amount of the compound inhibitor formulated in a carrier suitable for the chosen mode of administration. Administration is repeated daily for the duration of the test period. Beginning on day 0, cognitive and memory tests are performed, for example, once per month.

Patients administered the compound inhibitors are expected to demonstrate slowing or stabilization of disease progression as analyzed by changes in one or more of the following disease parameters: A beta present in CSF or plasma; brain or hippocampal volume; A beta deposits in the brain; amyloid plaque in the brain; and scores for cognitive and memory function, as compared with control, non-treated patients.

Example H

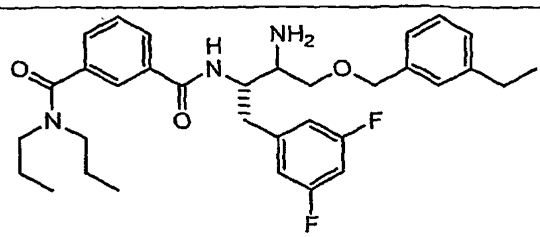
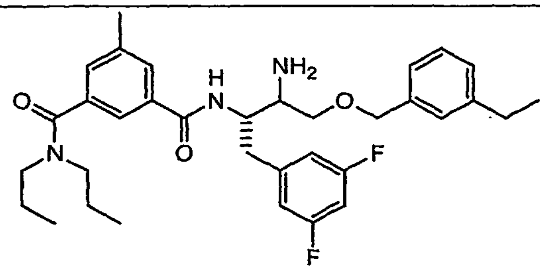
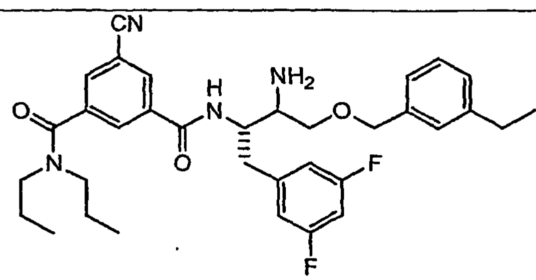
Prevention of A Beta Production in Patients at Risk for AD

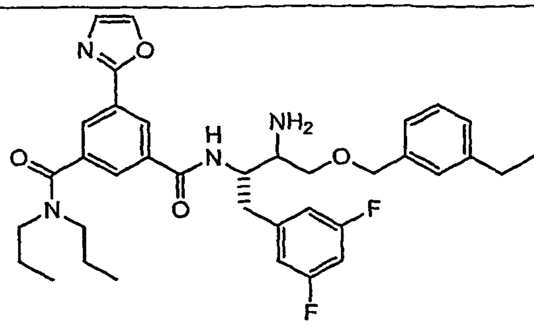
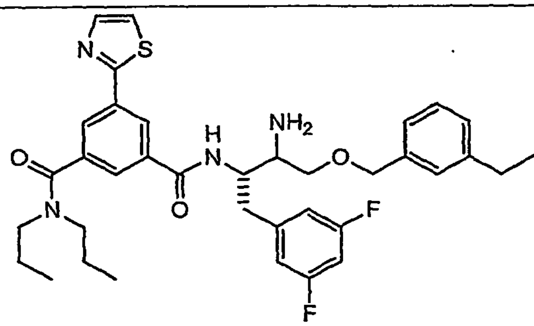
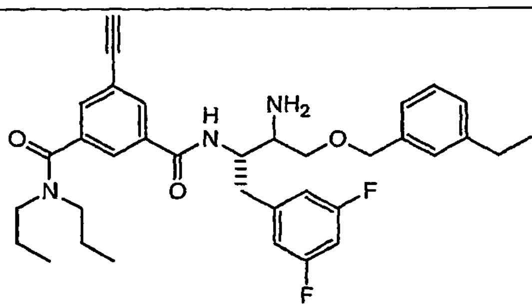
Patients predisposed or at risk for developing AD are identified either by recognition of a familial inheritance pattern, for example, presence of the Swedish Mutation, and/or by monitoring diagnostic parameters. Patients identified as predisposed or at risk for developing AD are administered an amount of the compound inhibitor formulated in a carrier suitable for the chosen mode of administration. Administration is repeated daily for the duration of the test period. Beginning on day 0, cognitive and memory tests are performed, for example, once per month.

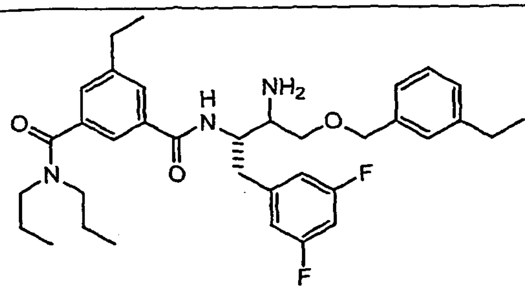
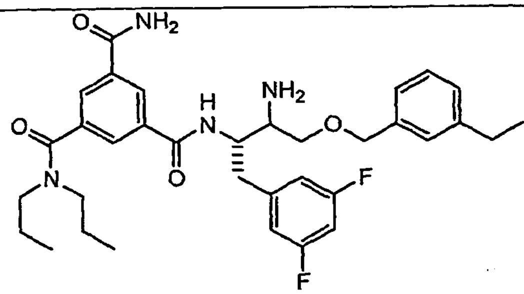
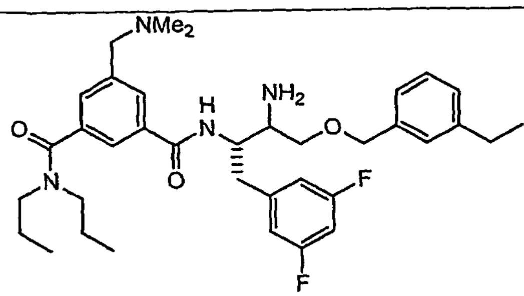
Patients administered the compound inhibitors are expected to demonstrate slowing or stabilization of disease progression as analyzed by changes in one or more of the following disease parameters: A beta present in CSF or plasma; brain or hippocampal volume; amyloid plaque in the brain; and scores for cognitive and memory function, as compared with control, non-treated patients.

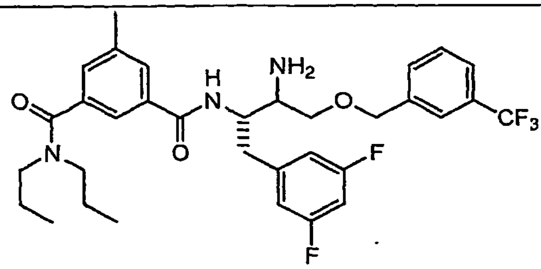
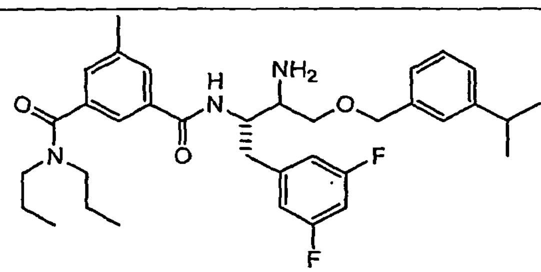
CHEMISTRY EXAMPLES

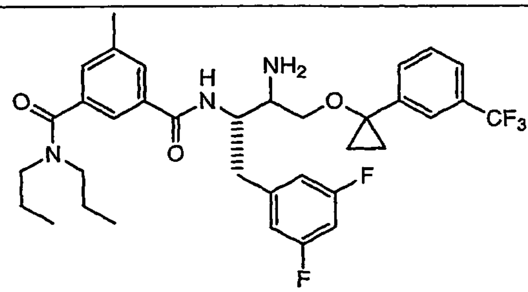
The following compounds are prepared essentially according to the procedures outlined above. All structures were named using Name Pro IUPAC Naming Software, version 6.00, available from Advanced Chemical Development, Inc., 90 Adelaide Street West, Toronto, Ontario, M5H 3V9, Canada.

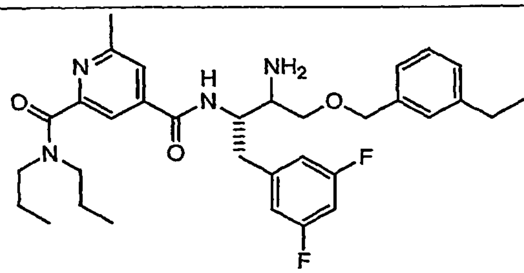
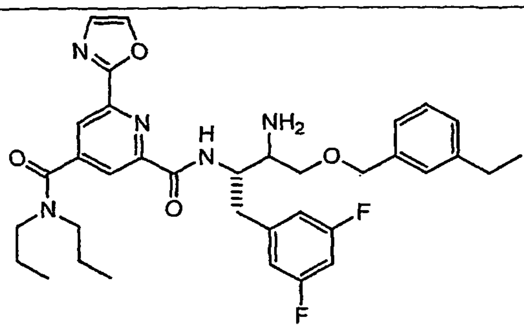
Ex. No.	Structure/Name
1	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-*N*,*N*-dipropylisophthalamide
2	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide
3	*N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-bromo-*N*,*N*-dipropylisophthalamide
4	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-cyano-*N*,*N*-dipropylisophthalamide

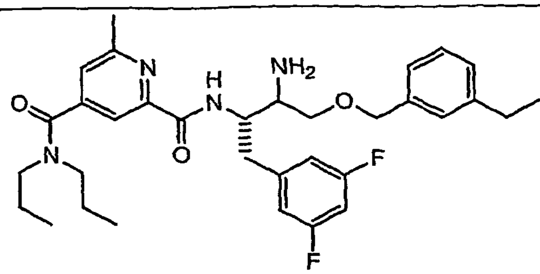
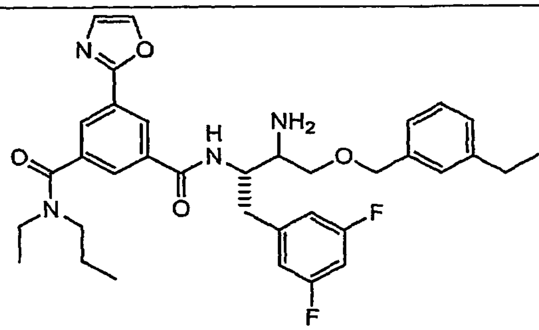
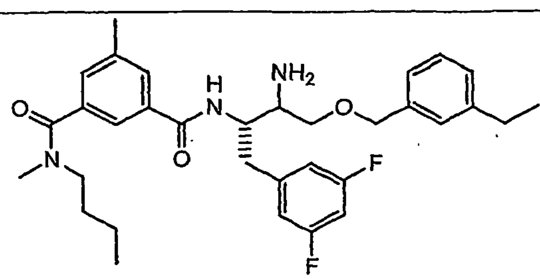
5	 *N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-(1,3-oxazol-2-yl)-*N,N*-dipropylisophthalamide
6	 *N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-*N,N*-dipropyl-5-(1,3-thiazol-2-yl)isophthalamide
7	 *N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-ethynyl-*N,N*-dipropylisophthalamide

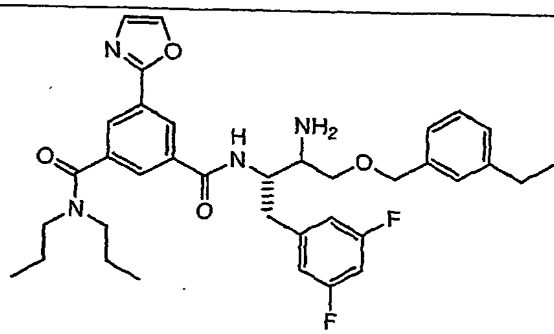
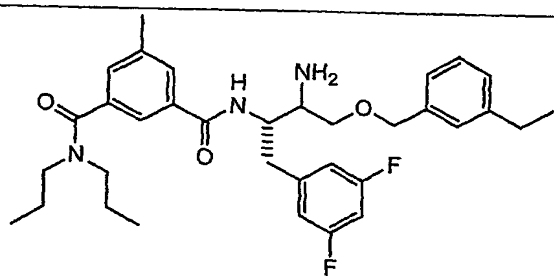
8	 N'-{(1S)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-ethyl-N,N-dipropylisophthalamide
9	 N^3-{(1S)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-N^1,N^1-dipropylbenzene-1,3,5-tricarboxamide
10	 N'-{(1S)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-[(dimethylamino)methyl]-N,N-dipropylisophthalamide
11	N'-{(1S)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-

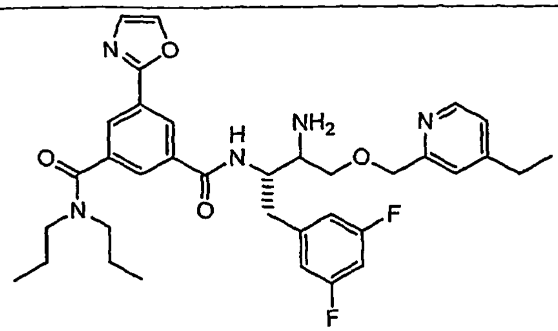
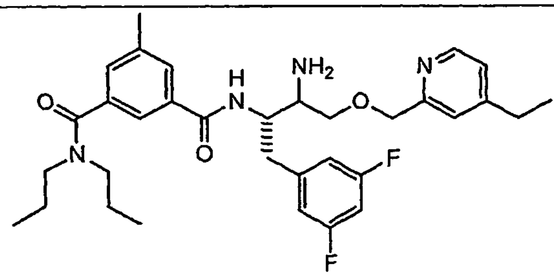
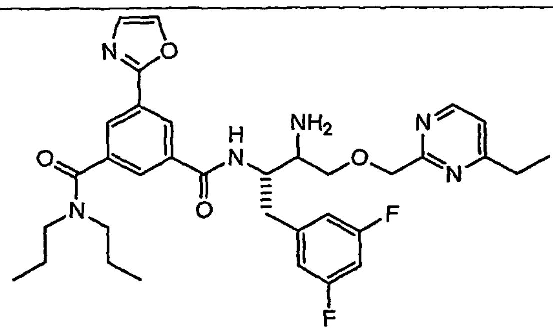
	ethynylbenzyl)oxy]propyl}-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
12	*N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethynylbenzyl)oxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide
13	*N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[3-(trifluoromethyl)benzyl]oxy}propyl)-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
14	 *N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[3-(trifluoromethyl)benzyl]oxy}propyl)-5-methyl-*N*,*N*-dipropylisophthalamide
15	*N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-isopropylbenzyl)oxy]propyl}-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
16	 *N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-isopropylbenzyl)oxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide
17	*N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-methoxybenzyl)oxy]propyl}-5-(1,3-oxazol-2-yl)-*N*,*N*-

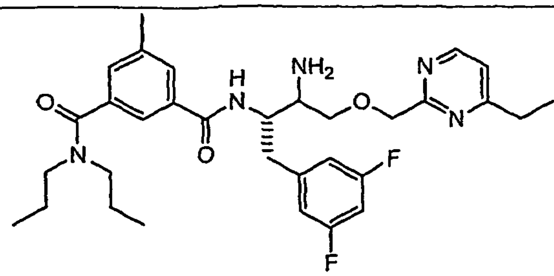
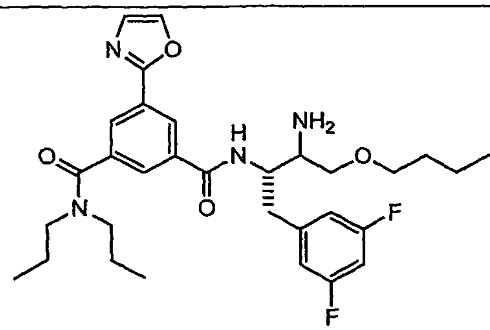
	dipropylisophthalamide
18	*N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-methoxybenzyl)oxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide
19	*N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-ethynylphenyl)cyclopropyl]oxy}propyl)-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
20	*N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-ethynylphenyl)cyclopropyl]oxy}propyl)-5-methyl-*N*,*N*-dipropylisophthalamide
21	*N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-({1-[3-(trifluoromethyl)phenyl]cyclopropyl}oxy)propyl]-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
22	 *N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-({1-[3-(trifluoromethyl)phenyl]cyclopropyl}oxy)propyl]-5-methyl-*N*,*N*-dipropylisophthalamide
23	*N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-isopropylphenyl)cyclopropyl]oxy}propyl)-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
24	*N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-isopropylphenyl)cyclopropyl]oxy}propyl)-5-methyl-*N*,*N*-dipropylisophthalamide
25	*N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-methoxyphenyl)cyclopropyl]oxy}propyl)-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
26	*N'*-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-

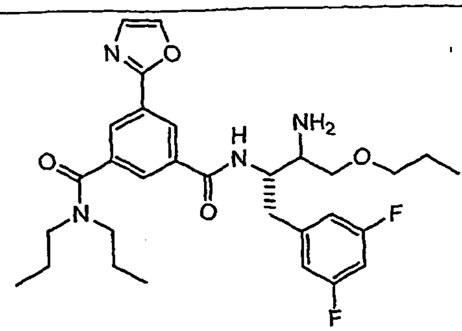
	methoxyphenyl)cyclopropyl]oxy}propyl)-5-methyl-*N*,*N*-dipropylisophthalamide
27	*N*'-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-ethylphenyl)cyclopropyl]oxy}propyl)-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
28	*N*'-((1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-{[1-(3-ethylphenyl)cyclopropyl]oxy}propyl)-5-methyl-*N*,*N*-dipropylisophthalamide
29	N^4-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-6-(1,3-oxazol-2-yl)-N^2,N^2-dipropylpyridine-2,4-dicarboxamide
30	 N^4-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-6-methyl-N^2,N^2-dipropylpyridine-2,4-dicarboxamide
31	 N^2-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-6-(1,3-oxazol-2-yl)-N^4,N^4-dipropylpyridine-2,4-dicarboxamide

32	 N^2-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-6-methyl-N^4,N^4-dipropylpyridine-2,4-dicarboxamide
33	 *N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-*N*-ethyl-5-(1,3-oxazol-2-yl)-*N*-propylisophthalamide
34	*N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-*N*-ethyl-5-methyl-*N*-propylisophthalamide
35	*N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-*N*-butyl-*N*-methyl-5-(1,3-oxazol-2-yl)isophthalamide
36	

	N'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-*N*-butyl-*N*,5-dimethylisophthalamide
37	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
38	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(3-ethylbenzyl)oxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide
39	*N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(6-ethylpyridin-2-yl)methoxy]propyl}-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
40	*N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(6-ethylpyridin-2-yl)methoxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide

41	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(4-ethylpyridin-2-yl)methoxy]propyl}-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
42	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(4-ethylpyridin-2-yl)methoxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide
43	 *N*'-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(4-ethylpyrimidin-2-yl)methoxy]propyl}-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide

44	 *N'*-{(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-[(4-ethylpyrimidin-2-yl)methoxy]propyl}-5-methyl-*N*,*N*-dipropylisophthalamide
45	 *N'*-[(1*S*)-2-amino-3-butoxy-1-(3,5-difluorobenzyl)propyl]-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
46	*N'*-[(1*S*)-2-amino-3-butoxy-1-(3,5-difluorobenzyl)propyl]-5-methyl-*N*,*N*-dipropylisophthalamide
47	*N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-(3-methylbutoxy)propyl]-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
48	*N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-(3-methylbutoxy)propyl]-5-methyl-*N*,*N*-dipropylisophthalamide

49	 *N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-propoxypropyl]-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
50	*N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-propoxypropyl]-5-methyl-*N*,*N*-dipropylisophthalamide
51	*N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-isobutoxypropyl]-5-(1,3-oxazol-2-yl)-*N*,*N*-dipropylisophthalamide
52	*N'*-[(1*S*)-2-amino-1-(3,5-difluorobenzyl)-3-isobutoxypropyl]-5-methyl-*N*,*N*-dipropylisophthalamide

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.